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THE UNIVERSITY OF ALBERTA

BACTERIA INVOLVED IN THE ROOT ROT DISEASE COMPLEX OF ALFALFA

by

© KAREN M. E. EMDE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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Date December 12, 1983

DEDICATION

To my family and friends.

ABSTRACT

In central Alberta a condition known as "alfalfa sickness" has been observed. The disease symptoms are as follows:

1. Plants are stunted and chlorotic.
2. Lateral and top roots exhibit irregular brownish lesions, Lateral roots are often girdled.
3. Poor nodulation is evident.

The pythiaceous fungus, *Phytophthora megasperma* has been shown to be a significant contributor to the disease process. During the course of this study it was determined that a large number of common soil bacteria play a significant role as co-pathogens. These organisms, studied in detail, include *Bacillus polymyxa, Chromobacterium violaceum, Clostridium* sp., *Cytophaga johnsonae, Erwinia carotovora* subsp. *carotovora, Lysobacter enzymogenes, Pseudomonas fluorescens, Pseudomonas marginalis* and *Serratia* sp. All were isolated from lesioned alfalfa roots collected in the field. These isolates were subsequently able to re-infect and cause necrosis in alfalfa seedlings, mature alfalfa root sections, canola seedlings and sterile discs of carrot, potato and onion tissue.

Bacterial alfalfa pathogens found in this study are not invasive and require a "wound" prior to entry into the alfalfa root. An important natural wounding mechanism is primary invasion by *P. megasperma, Fusarium* sp. and other soil fungi. Other mechanisms of wounding, that may contribute, are feeding of nematodes and alternate freezing and thawing of roots during the course of the winter.

"Alfalfa sickness" should be thought of as a disease complex involving the interaction between soil fungi, nematodes and bacteria as well as the interaction between the plant roots, the environment and the pathogen.

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I. INTRODUCTION

Root rot of alfalfa *(Medicago sativa)* in north-central Alberta is not a new problem. It was first described in 1962 when it was noticed that yields of alfalfa hay grown on soils previously cropped with alfalfa were lower than yields from soils growing alfalfa for the first time (Geottel, 1962). Growth in these fields was variable with the majority of plants showing symptoms of stunted growth, poor nodulation and chlorosis (Goettel, 1962; Webster and DeKock, 1970). Examination of roots revealed irregular brown to black lesions on lateral and tap roots, often followed by girdling of the lateral roots (Damirgi *et.al.*, 1976).

Greenhouse and field studies showed that neither nutrient deficiencies or toxicity were responsible for this condition (Goettel, 1962; Webster *et.al.*, 1967; Webster and DeKock, 1970). Sterilizing infested soils with steam and/or chemicals produced beneficial effects on alfalfa growth indicating a biological agent was responsible (Webster *et.al.*, 1967).

Studies by Webster *et. al.* (1972) and Webster and Hawn (1973) indicated that *Paratylenchus projectus,* a soil nematode, was abundant in fields of "diseased" alfalfa. Further study showed that *P. projectus* alone was not responsible for alfalfa stunting (Damirgi *et.al.*, 1976). These studies showed that root lesioning would only occur when *P. projectus* was combined with dilutions of "sick alfalfa soil. Isolations from these lesions yielded *Phytophthora megasperma* (Damirgi *et.al.*, 1978), a known root-rot fungal pathogen (Erwin, 1965). *P megasperma* was cited as one of the biological agents responsible for alfalfa sickness.

The concept of rhizosphere is not new and is used to describe that region of soil under the direct influence of plant roots (Alexander, 1977,; Lochhead, 1958). This region has an abundant and diverse microflora associated with it and there are many ways, microscopic, cultural or biochemical to study its behaviour (Alexander 1977). The bacterial component of the rhizosphere microflora is enormous and these bacteria are nutritionally complex and diverse. Because of the preferential stimulus due to plant roots in the rhizosphere over non-rhizosphere soils, the bacterial population of the rhizosphere is more active than in a non-rhizosphere soil. In the rhizosphere there are higher proportions of cellulolytic, proteolytic and saccherolytic organisms as well as those responsible for mineral cycling in soil.

The microbial community of the rhizosphere has been thought as consisting primarily of non-pathogenic organisms and saprophytes (Lochhead, 1958; Alexander, 1977). Phytopathogens were described as "invaders" which implied penetration and invasion of the rhizosphere. The physiological response of roots to this invasion would then modify the rhizosphere microflora and the pathogen would be either successful or be suppressed. The plant would then be termed resistant or susceptible to the pathogen. This theory will hold true for many phytopathogens but not all. Because of the nutritional and physiological complexity of soil bacteria, many possess mechanisms needed to utilize plant tissue components. However most soil bacteria lack the ability to invade intact plant tissues and must depend on a second variable to provide a means of entry. Once inside the plant these organisms can utilize the array of nutrients which the plant tissue provides. Cell components are degraded

and the integrity of the plant cell is lost. Such bacteria would be called opportunistic phytopathogens.

The objectives of this present study was, then, to determine if bacteria found in lesioned alfalfa roots were indeed phytopathogens, to characterize and classify these bacterial pathogens and to prove their pathogenicity to alfalfa.

II. LITERATURE REVIEW

Early observations

The concept that bacteria are able to cause plant diseases has had a long and, at times, confusing history. Early reports of soft-rot organisms were not always well documented and many researchers experienced difficulties in the taxonomy of these organisms. This was due primarily to the disarray that existed in the methods of bacterial taxonomy in the early part of this century.

One of the first documented references to soft-rot bacteria was published in 1886 by C.J. Davaine (Ainsworth, 1981) who published short notes on the rotting of fruit. He showed that these symptoms were easily transferred from spoiled fruit to fresh fruit and called the organism *Bacillus termo*, which may in fact be *E. carotovora*. In 1901 Jones described *Bacillus carotovorus (E. carotovora)* and demonstrated its ability to cause soft-rot in many fleshy vegetables and plant parts (Burkholder and Smith, 1949). *Bacillus atrosepticus (E. atroseptica)* was described in 1902 by van Hall and shown to be the causal agent of potato blackleg (Burkholder and Smith, 1949). Similar history can be found for the other well-known bacterial phytopathogens, *P. fluorescens*, *P. marginalis* and *B. polymyxa*.

Because at that early time there were not many set taxonomic procedures for bacterial classification, confusion developed with classification of the bacterial phytopathogens. Taxonomic methods used were generally those developed for medical microbiology. The host from which the pathogen was isolated played an important role in taxonomy as the organisms were thought to be host-specific. For example, *Pseudomonas ananas* (Elliott, 1951) was isolated from a soft-rot of pineapple.

Names such as these were dropped from modern taxonomic references as many organisms were found to be the same even though they may have been isolated from different hosts. Thus *P. ananas* is in fact *P. fluorescens*. For these reasons a great number of "new" diseases were described. The novelty in fact was that the host plant had not been previously described as being affected by bacteria.

Fungi at this time received considerably more attention, primarily because they were easier to classify. Fungi are larger, more striking in appearance and have distinct characteristics in hyphae, sexual and asexual spores, lifecycle and are more aggressive in pursuit of their chosen host. Because fungi can be easily isolated from most plant infections and they overwhelm the bacteria present, this led to the view that fungi cause most plant diseases while bacteria appeared to be rather insignificant. A common viewpoint was expressed by Burkholder (1949); "Bacterial plant pathogens are not soil-inhabiting organisms and are apparently unable to stand the competition in nature.". Even today this viewpoint is still widely accepted. Many pathologists cannot visualize bacterial phytopathogens existing merely as soil microorganisms without a host or plant debris in a non-parasitic phase. Many phytopathogens also play an important part in the soil environment such as in mineral and nutrient cycling. Failure to recognize this leads one to a rather limited view of bacteria as phytopathogens and as soil inhabitants. These restrictions limit the viewpoint as to what constitutes a root pathogen and what does not. Dommergues (1978) expressed the view that; "relatively few species of bacteria are root pathogens, these being aerobic, non-spore forming rods in the genera *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas*

and *Xanthomonas."*

Disease Process

In order to understand how soft-rot bacteria cause necrosis, it is first necessary to understand the basic plant cell structure and the means pathogens have to attack plant cells. All plant cells have a basic common structure. The protoplast has the potential to develop into various plant tissues, depending on the environment and the requirements to survive in that environment (Scott, 1978).

The basic plant cell consists of major amounts of cellulose, pectic polysaccharides, hemicellulose, structural glycoproteins, lignin and minor amounts of waxes, terpenes, fats, alkaloids, amino acids, tannins and phenols (Codner 1971; Mount, 1978; Scott, 1978). The proportions of these ingredients change as the plant ages. The cell wall consists primarily of cellulose fibers interspersed with pectic acids, hemicellulose, lignin and is coated with mucilage. The pectic acid fraction in the primary cell wall is thought to be arranged in chains of an α-1,4-linked galacturonan polymer. The hemicelluloses are covalently linked by glycosidic bonds at the reducing ends of the pectins and are linked by hydrogen bonds to the cellulose fibers. There is also a carbohydrate rich glycoprotein consisting of major amounts of arabinose and galactose, that is linked to the pectic acid fraction and may aid in stability (Albersheim *et. al.* 1969; Zucker *et. al.* 1972; Basham & Bateman, 1974; Mount *et. al.* 1978). The secondary cell wall consists mainly of cellulose, pectins, hemicellulose and lignins. Between the primary and secondary walls is the region known as the middle lamella. It is composed primarily of pectic acids but as the plant ages, the middle lamella often becomes lignified (Esau, 1977).

In the laboratory studies of cell injury by pectic enzymes injury is illustrated by the inability of cells to accumulate Neutral Red, a vital stain. There is also an increase in loss of solutes and water from the cell and a loss of all ability to plasmolyse in hypertonic solutions (Tribe 1955; Mount *et. al.* 1970; Hall and Woods 1973).

In order for a phytopathogen, bacterial or fungal, to be successful in breaching this formidable plant cell wall it must possess the enzymes able to degrade cell wall constituents. With soft-rot organisms one group of enzymes are collectively called pectic enzymes; hydrolases and transeliminases (Codner 1971; Zucker *et. al.* 1972; Mount *et. al.*1978). Both types cleave the 1,4-glycosidic bonds in the pectin polymer. Hydrolytic pectic enzymes include pectin methylgalacturonase and polygalacturonase, while transeliminative pectic enzymes include pectin methyltranseliminase and polygalacturonate transeliminase (Codner 1971; Mount *et. al.* 1978). Both types of enzymes are able to cause cell damage but the mode of action is different. Hydrolases cleave the α-1,4 bond of galacturonic acid chains yielding a reducing group and a terminal nonreducing end. Trans-eliminases cleave the same bond but instead produce a reducing group and a β-4,5 unsaturated uronide (Bateman and Millar, 1966). These enzymes are further characterized by the manner in which they attack pectin/pectic acid, that a random cleavage or -endo enzyme of a terminal cleavage or an -exo enzyme (Codner 1971; Hildebrand 1972).

In addition to the above pectic enzymes a third enzyme, pectin methylesterase, is also found in many soft-rot bacteria. This enzyme is a hydrolase but its mode of action is different. Rather than cleaving the pectin polymer, pectin methylesterase removes the methoxy groups of the pectin molecule yielding pectic acid (Codner 1971; Bateman and

Miller 1966). Bateman and Millar (1966) proposed that the presence of methylesterase may be important in stimulating the activity of pectin transeliminases as pectic acid is the preferred substrate of these enzymes.

As stated before all soft-rot bacteria produce some type of pectic enzymes in varying amounts. Zucker *et. al.* (1971) subdivided the soft-rot organisms into three groups based on the amount of enzyme produced. Organisms with a high activity of extracellular pectate lyase include *E. carotovora, E. aroidae, E. atroseptica, P. fluorescens* and *P. marginalis.* Other organisms shown to produce comparable amounts of extracellular pectate lyase include *B. polymyxa* (Nagel and Wilson, 1970), *Flavobacterium* sp. and *Clostridium* sp. (Lee *et. al.* 1970; Pérombelon *et. al.* 1973; Lund and Brocklehurst 1978; Cabezas de Herrera and Sanchcz Maeso 1980). Christensen (1977) suggested that *Flavobacterium pectinovorum* is more properly classified as Cytophaga johnsonae.

Regulation of these enzymes is multifaceted. One important factor is pH, which can be used to distinguish between enzymes. Polygalacturonase has a pH optimum of pH 5.0 while the optimum for pectate lyase is pH 8.0. The use of polypectate gels at different pH values takes advantage of this difference and allows one to assess which enzyme is active (Hildebrand 1971; Zucker *et. al.* 1972).

The substrate available is important in regulation of enzyme synthesis. Soft-rot *Erwinia* sp. have an inducible synthesis of pectate lyase, while the pseudomonads are subject to constitutive control (Zucker *et. al.*1972). Addition of polypectate or pectin to media supporting *Erwinia* sp. increased pectate lyase activity over that of media containing glucose or glycerol (Zucker *et. al.* 1972).

Pseudomonads showed higher pectate lyase activity when grown on media containing glucose or glycerol and appeared unable to metabolize the degradation products of pectin unless potato extract was added (Zucker *et. al.* 1972). This suggested that catabolite repression by glucose may be operating, as lyase activity increased fourfold when the organism was grown on glycerol rather than glucose.

Pectic enzymes are not the only weapon of bacterial phytopathogens. Many organisms also possess enzymes able to hydrolyze galactans (galactanases, galactosidases) and arabans (arabinases). Cellulases are produced by some phytopathogens, namely *B. polymyxa*, *Clostridium*, and *Cytophaga* sp. Because of the large percentage of cellulose in plant cell walls, the ability of an organism to produce cellulase makes it a much more efficient and competitive pathogen. Work by Beraha and Garber (1971) and Friedman (1962, Friedman and Ceponcs 1959) suggest that mutants of *E. carotovora* and *Pseudomonas* unable to produce extracellular pectate lyase, also lack other enzymes such as cellulases and proteases. They further suggest that these mutations may alter regulatory mechanisms responsible for enzyme synthesis or they may alter bacterial cell walls and restrict secretion of the enzymes.

Injury to plant

The most visible signs of a soft-rot infection are maceration and rotting of the parenchymatous tissue of the affected part. Maceration of the parenchymatous tissue has been shown to be initiated by a single enzyme (Bateman and Basham 1976). A purified polygalacturonate transeliminase of *Erwinia chrysanthemi* was shown to macerate potato tissue (Bateman and Basham 1976). This ability combined with that of the other bacterial enzymes attacking cell wall constituents provides

a formidable arsenal for bacterial phytopathogens.

In plant cells damaged by pectic enzymes there is a phenomenon of rapid cell death. This is characterized by the loss of ability of cells to accumulate Neutral red. Such cells exhibit an increase in electrolyte loss and a loss of ability of cells to plasmolyse in hypertonic solutions (Hall and Woods 1973; Basham and Bateman 1974), and a loss of the ability of the plasmalemma to act as a semipermeable barrier. It is thought unlikely that pectic enzymes act directly on the plasmalemma (Hall and Woods 1973). Basham and Bateman (1974) propose that in a polygalacturonate transeliminase treated cell, the internal turgor pressure forces the plasmalemma tightly against the cell wall. This pressure can cause rifts in the cell membrane destroying its integrity. Under such conditions the plasmalemma exhibits increased permeability to ions and may in fact burst due to the increase in osmotic pressure (Wood 1976).

Other enzymes produced by soft-rot bacteria that can contribute to the death of protoplasts are proteinases and phosphidases (Wood 1976). They may contribute by acting on components of the plasmalemma or other plant cell membranes. However when proteinases and/or phosphidases were put on unplasmolysed discs of potato tubers, they could not cause cell separation or death of protoplasts (Wood 1976). This would imply that an interaction between many enzymes is necessary to cause cell death.

Disease complexes

As mentioned previously bacteria were thought to play a rather limited role in root diseases. Widespread acceptance of the role of bacteria in these diseases was hampered by the general confusion in the taxonomic status of bacterial phytopathogens. Contributing to the

uncertain status of bacterial phytopathogens were the lack of specificity of many taxonomic methods, the use of selective media that was often unable to detect low populations of organisms and a general lack of understanding of how bacteria survive in a nonpathogenic state.

In order to appreciate the interaction between plant, environment and pathogens it is first necessary to understand the concept of a rhizosphere and the elements functioning within it. As mentioned previously the term rhizosphere is used to deliniate that region of soil under the direct influence of plant roots. Implicit in this definition is the fact that microorganisms are more abundant in this region than in root-free soil. The numbers and types of organisms change with changes in the plant root system. Thus the maximum rhizosphere effect is reached at the stage of maximum vegetative growth of the plant (Lochhead 1958; Buddenhagen 1965; Schroth *et. al.* 1979).

The components of the rhizosphere do not function as separate entities, but interact with each other and may be dependant on the activity of other components. In its simplest form this relationship can be thought of as a pyramid with the plant (host), the soil environment, soil microorganisms and time at each corner of the pyramid (Bateman 1978). When one of these components is altered, the other factors adjust to accommodate the change. This reasoning favors a multicomponent hypothesis of parasitism (Bateman 1978). Implicit in this hypothesis is that the host and pathogen have a favourable and an unfavourable environment in which to interact. In each case these environments are made up of a number of components and it is the total sum of all the components that determines the type of host-pathogen interaction. For example, should conditions be such that a pathogen

can gain entry into and cause disease symptoms in the host, then this "sum of components" would be weighted for a favourable environment to the pathogen (Bateman 1978).

With respect to physical factors influencing disease it has been well documented that cool, wet conditions are conducive to root rot (Goto 1972; Lund 1979; Molina and Harrison 1980; Pérombelon 1980). Prolonged wet conditions inhibit free diffusion of oxygen within the soil matrix thus limiting the amount of available oxygen to plant roots and soil microflora (Drew and Lynch 1980). This has a significant effect on microbial activity at the roots and a competition for oxygen ensues between roots and microorganisms. Shortage of oxygen and a surplus of water causes stress to the plant and it manifests this stress in several ways. Photosynthetic activity decreases under conditions of water stress and cooler temperatures. This results in a decrease in exudation from roots which in turn reduces the amount of energy sources available to rhizosphere microorganisms.

One aspect of root damage that has received considerable attention is damage due to freezing (Jones 1928; Steponkus 1978). This type of injury becomes significant in the northern temperate regions of the world. The degree of injury the plant sustains depends on soil factors such as compaction, tendency toward frost heaving, waterholding capacity, cultural practices, and climatic conditions for that time period. Perennial crops are bred to withstand low freezing temperatures However the alternate cycles of freezing and thawing to which a plant is subjected throughout the course of the winter can cause considerable stress to the root system and may be a significant factor in creating avenues for non-invasive phytopathogens to enter root systems.

Larger soil organisms such as fungi and nematodes play a significant role in aiding bacterial phytopathogens to gain entry into root systems. It is well known that nematodes comprise a significant segment of the plant rhizosphere population and that they feed on plant roots (Agrios 1978) or plant debris (Bookbinder *et. al.*,1979). Not all nematodes cause disease symptoms on the host plant merely by the act of feeding. However in many root-rot diseases significant numbers of nematodes have been observed and it is not always immediately obvious what role they play in the disease process. Many saprozoic nematodes are able to feed on bacteria or bacterial by-products and are thought to be a means of introducing bacterial pathogens into the plant root (Kalinenko 1936; Chantanoa and Jensen 1969). The nematode *Pristionchus iheritieri* was shown to feed on *Agrobacterium tumefaciens, Erwinia amylovora, E. carotovora, Pseudomonas phaseolicola* and *Serratia marcesens*. These bacteria were able to survive passage through the nematode and produce viable colonies on nutrient agar (Chantano and Jensen 1969). They also observed that bacteria were able to adhere to the nematode body surface, which could serve as an additional method of dissemination.

Kalinenko (1936) showed that bacteria could be injected into the root system by nematodes feeding on these roots. He used two species of rubberplant, *Taraxacum kok-saghys* and *Scorzoneratau saghys* and found the following nematodes in the roots; *Tylenchus multicincta, Tylenchus pratensis* and *Aphelenchus avenae*. In the intestinal tract of these nematodes Kalinenko found *E. carotovora, Pseudomonas phaseoli, P. fluorescens, Bacillus mesentericum and Proteus vulgaris*. When rubberplant roots were inoculated with bacteria isolated from the intestinal tract of the nematodes, these bacteria were able to cause

symptoms of necrosis and tissue maceration. Kalinenko postulated that the combined action of the nematode plus bacteria caused extensive root lesioning with the nematode providing the mechanical injury and the bacteria providing the enzymatic injury.

Fungi have been shown to be associated with and be responsible for many root rot diseases. They are extremely aggressive pathogens and can initiate infection easily. Chi and Childers (1966) showed that *Phythium* and *Phytophthora* reduced emergence of, andcaused damping off of alfalfa in eastern Ontario. They also found that in the same alfalfa fields *Fusarium* and *Rhizocotina* were able to incite wilt and root rot. A seasonal distribution of fungi was postulated which appeared to correlate with the optimum temperature for growth of these fungi. As the season advanced *Phythium* and *Phytophthora* species decreased in numbers while *Fusarium* and *Rhizocotina solani* increased.

Many investigators report that bacteria appear to be associated with some fungal root rot diseases (Nielson 1949; Bushong and Gerdemann 1959; Stanghellini 1972; Gaudet *et. al.* 1980). Bushong and Gerdemann (1959) reported that *Phytophthora* was most often isolated from alfalfa in early spring and fall while *Fusarium,* in combination with other soil fungi and bacteria was isolated during summer. This would appear to support the theory of a seasonal distribution of pathogens.

Fusarium species have been reported to be in association with blackleg of potatoes (Nielson 1949; Stanghellini 1972), root and crown rot of sainfoin (Gaudet *et. al.* 1979) and bottom rot of lettuce (Pieczarka and Lorbeer 1974). *Fusarium roseum* was shown by Stanghellini (1972) to "activate" the soft-rot bacteria isolated from infected potato seed tubers. That is, tubers containing both *F. roseum* and soft-rot

bacteria exhibited a greater degree of necrosis and tissue maceration than those tubers containing only soft-rot bacteria or only *F. roseum*.

Root and crown rot of sainfoin (*Onobrychis viciaefolia* Scop.) appear to share many symptoms with alfalfa root rot (Gaudet *et. al.* 1980). Early investigators believed that *Fusarium solani* was the primary causal agent, however the inability of researchers to consistently isolate *F. solani* from infected plants lead to further investigation. Bacteria were isolated from three and four year old plants and found to be restricted to the vessels and broken cavities in the xylem. Cellular decay was associated with the presence of bacteria in these regions. These bacteria were classified as *Pseudomonas syringae*, *Pseudomonas marginalis (P. fluorescens)* and an *Erwinia amylovora*-like organism. Gaudet and his co-workers (1980) concluded that root and crown rot of sainfoin in Montana appeared to be caused by a group of bacteria rather than *F. solani*.

Pieczarka and Lorbeer (1974) found that bottom rot of lettuce (*Lactuca sativa*) appeared to be a disease complex with both a fungal and a bacterial component. Bottom rot of lettuce was first described as being caused by *Rhizoctonia solani*. However due to the rapid slimy decay in the advanced stages of bottom rot, a complex involving bacteria and/or decay fungi and *R. solani* was postulated. These organisms would most likely invade the host after initial infection by *R. solani*. During the course of bottom rot *R. solani* in the more advanced stages indicating bacteria are significant as secondary pathogens. Bacterial pathogens isolated from advanced stages of bottom rot were *E. carotovora*, *P. fluorescens* and *P. marginalis*. Bacteria appear to modify symptom development of bottom rot when they are compared to plants inoculated only with *R. solani*.

Survival of soft-rot bacteria

Much has been written about what soft-rot bacteria do when in contact with suitable hosts, however the way in which these organisms survive their non-pathogenic state and how they are able to persist in soil is not yet that well understood by plant pathologists. Many early investigators favoured the notion that most bacterial phytopathogens were unable to overwinter in soil. Such conclusions were based on results from use of selective media which were not overly sensitive to low populations in the soil. A point to keep in mind when evaluating some of these early observations is that negative results can be misleading. Failure to detect an organism on a particular medium cannot be thought of as absolute proof of its absence. It may only mean that the medium or methods used were not sensitive enough for low endemic populations or that the conditions for isolation were not met.

With the multiplicity of microbial habitats in the soil and the soil and the variety of energy sources available, it is inconceivable that bacterial phytopathogens are unable to exist if the host is not present. Many other plants, especially volunteer plants of the affected crop, noxious weeds or other cultivated plants can serve as reservoirs of bacterial phytopathogens.

Kikumoto and Sakomoto (1969) observed that a variety of plants were able to support the growth of *E. carotovora* in the field. Especially significant was their finding that soft-rot bacteria were a component of many common weeds growing in fields of chinese cabbage *(Brassica chinensis): Agrostis perennans, Portulaca oleracea, Sonchus oleraceus, Chenopodium album* and *Commelina communis*. They also showed lower populations of *E. aroideae* in the rhizospheres of radish, red bean, wheat and tomato.

Pseudomonads have also been reported to frequent rhizospheres of plants other than their immediate hosts (Goto 1972; Rovira and Sands 1971; Valleau *et. al.* 1944). Valleau and his workers showed that *Pseudomonas angulata* was able to overwinter in the rhizospheres of several winter-cover crops and associated weeds: crimson clover, vetch, barley, wheat, rye and chickweed.

Xanthomonas oryzae and *Xanthomonas vesicatoria* have both been shown to overwinter on wheat, common grasses and weeds (Valleau *et. al.* 1944; Isaka 1969; Claflin and Stuteville 1973). Isaka's work with this pathogen showed that *X. oryzae* was most common on aerial portions of *Bromus unioloides, Phalaris arundinaceae* and *Alopecurus fulvus*. There was carry-over on *Trifolium repens, Astragalus sinicus* and *Rumex japonicus* which suggested that these weeds were also important sources of inoculum. It was not reported whether any of these bacterial pathogens were able to incite disease symptoms in these alternate non-hosts. Goto (1972) concluded that population levels of these pathogens in the non-host phase may represent their degree of saprophytic ability. Another factor to consider is survival on plant debris within and on the soil. This is often considered an important source of inoculum. A third point to consider is that all these bacterial pathogens can be grown on chemical media within a laboratory setting and survive very well. The ability to incite soft-rot symptoms may disappear after frequent cultivation in the absence of a suitable plant host, however this could be seen as evidence for the inducible nature of pectic enzyme production on soft-rot bacteria.

III. MATERIALS AND METHODS

More than two hundred root-rot bacteria were isolated from lesioned alfalfa roots. A small number were obtained from weeds growing in association with alfalfa, which also had necrotic areas on their roots. The alfalfa roots were collected from fields in central Alberta, primarily the St. Paul-Mallaig area, Mayerthorpe, Morinville and Spruce Grove, Alberta. These fields all exhibited the aforementioned symptoms of alfalfa sickness.

Alfalfa roots were brought back to the laboratory and were kept at 5°C until they could be examined. Roots were rated for degree of lesioning using a 20X magnifying glass and a stereo microscope. A disease index was used to determine the degree of lesioning (Table 1) and is the one used by Damirgi *et. al.* (1976, 1978).

Table 1. Lesion rating scale.

Rating	Interpretation
1	No visible lesions; roots visably clean and healthy
2	Slight browning on exterior and/or interior of tap root (salt and pepper appearance), lateral roots or fine roots.
3	Moderate lesioning on exterior and/or interior of tap roots, lateral roots, fine roots or crown. May see minor girdling of lateral roots.
4	Extensive lesioning on the exterior and interior of tap roots, lateral roots and crown. Most fine roots are missing. Extensive girdling of lateral roots.
5.	Dead plant.

Necrotic areas were excised and surface-sterilized in 0.1% (w/v) $HgCl_2$ with two drops of Tween 80 added per 100 milliliters. This was followed by washing in 95% denatured ethanol and a thorough rinsing in sterile distilled water. The root sections were then aseptically macerated and serial dilutions made. Dilutions from 10^{-3} to 10^{-7} were plated onto Plate Count agar (Difco), B_{10} agar, Cereal agar, V_8 juice (Campbells) agar and Rose Bengal agar. Plates were incubated aerobically at 22°C for two to seven days and were examined daily for suspect colonies. These colonies were subcultured to Plate Count agar (P.C.A.) and purified. Pure isolates were screened for pathogenicity on sterile slices of wounded and unwounded carrots, onions and potatoes on water agar plates. The wounds were a series of small, shallow cuts made in the vegetable tissue. The vegetable slices were sterilized in the same manner as the alfalfa root tissue. Water agar plates proved to be better than moistened filter paper for keeping a constant humidity within the petri plates. The plates were incubated aerobically at room temperature and were examined daily for signs of necrosis (Lund 1972, 1979; de Mendonca and Stanghellini 1979; Pérombelon *et. al.* 1973).

Two different types of necrosis were evident. The first was a dark brown to black watery necrosis typified by *Erwinia carotovora* subsp. *atroseptica* and *Erwinia carotovora* subsp. *carotovora*. The second was an overall breakdown of tissue with only a very light brown coloring. This was most often seen with *Pseudomonas* species.

A number of known organisms were used as controls and are listed in Table 2. Negative control consisted of an uninoculated wounded and unwounded vegetable tissue incubated under the same conditions. Appendix 1 lists all the isolates and their source.

B. polymyxa, *B. subtilus*, *C. johnsonae*, *C. violaceum*, *E. carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora*, *Lysobacter*, and *P. fluorescens* proved to be quite active in rotting vegetable tissues and constitute a large portion of the phytopathogenic bacteria isolated from alfalfa roots.

Vegetable tissue pathogens were reisolated from necrotic vegetable tissue, checked for purity and maintained in Trypticase Soy Broth (Difco) and were transferred to fresh Trypticase Soy Broth (T.S.B.) every two weeks. These stock cultures were the source of inoculum for subsequent experiments. Periodic checks for purity were made by plating onto P.C.A. for single colonies. Pathogenicity was also checked periodically by inoculating sterile carrot and potato slices.

Table 2. Named organisms used as reference controls and their source.

Organism	Source
Bacillus polymyxa	Department of Soil Science Collection University of Alberta
Bacillus subtilus	Department of Soil Science Collection
Chromobacter violaceum	Department of Soil Science Collection
Corynebacterium insidiosum	Dr. R. Copeman, Department of Plant Science, University of British Columbia
Cytophaga johnsonae	Department of Soil Science Collection
Erwinia amylovora	Dr. Keil (deceased), Fruit Laboratory Beltsville Agriculture Research Center, Beltsville, Maryland, U.S.A.
*Erwinia amylovora***	Dr. F.L. Lukezic, Department of Plant Pathology, Pennsylvania State University, University Park, Pennsylvania
Erwinia carotovora subsp. *atroseptica*	Dr. R. Copeman
Erwinia carotovora subsp. *carotovora*	A.T.C.C.+ V73-103
Escherichia coli	Department of Soil Science Collection
Lysobacter sp.	Department of Soil Science Collection
Pseudomonas aeruginosa	A.T.C.C. 9027
Pseudomonas corrugata	Dr. F.L. Lukezic
Pseudomonas fluorescens	A.T.C.C. 17397
Pseudomonas syringae	Dr. A.W. Henry, Department of Soil Science, University of Alberta*
Serratia marcesens	Department of Soil Science Collection

+ American Type Culture Collection

* retired

** In 1981 this organism was subsequently described as *S. marcesens* (Dr. F.L. Lukezic, personal communication).

A. Identification

Most of the organisms isolated were either aerobes or faculative anaerobes. Only six isolates were strict anaerobes. Motility and gram stain reaction were observed on 24 hour cultures grown in Trypticase-soy broth (T.S.B.). Cultures that exhibited motility were stained with a flagella stain (British Biological Laboratory, B.B.L.). The type of flagellation was described as per Leifson (1960). Pigment production was determined on Plate Count agar (P.C.A.) plates and King's medium A plates (Corpe 1953; Hendrie and Shewan 1966; Park and Holding 1966; Rhodes 1959). Those colonies producing a yellow-green diffusable pigment on King's medium A were examined under ultraviolet light for their ability to fluoresce.

Oxygen requirements and ability to utilize glucose as a carbon source were determined by deep stab inoculations into duplicate tubes of Board and Holding medium plus glucose. Immediately after inoculation one of the tubes received a plug of sterile wax and both tubes were incubated at room temperature for 24 to 72 hours.

Ability to produce acid and/or gas from carbohydrates and related carbon sources was observed in liquid cultures of nutrient broth (Difco) with 0.5% (w/v) Seitz-sterilized carbohydrate or carbon source added after autoclaving. Phenol red was used as a pH indicator. Carbon sources used were arabinose, xylose, fructose, sucrose, mannose, maltose, lactose, cellobiose, raffinose, mannitol and ethanol. Inverted Durham tubes were placed in the test tubes to detect gas production. Tubes were incubated at room temperature for 24 to 72 hours (Edwards and Ewing 1972; Ewing 1962; Graham 1972; Park and Holding 1966).

Action on pectate was determined by using Crystal violet pectate agar (1N NaOH 4.5 ml, 10% $CaCl_2.H_2O$) 3.0 ml, Bacto agar 1.5 g, $NaNO_3$ 1.0 g, boiling distilled water 300 ml, sodium polypectate 15.0 g dissolved in 200 ml boiling distilled water, 0.075% (w/v) crystal violet 1.0 ml). pH was adjusted to pH 5.5, pH 6.9 and pH 8.0. Pectase action was noted by the formation of pits in the medium around the colony. Plates were incubated at room temperature and examined after 3, 7, 14 and 21 days (Burr and Schroth 1977; Cuppels and Kelman 1974; DeBoer *et. al.* 1978; Hankin *et. al.* 1971; Hildebrand 1971; Meneley and Stanghellini 1976).

To determine the presence of amino acid decarboxylases, Moeller Decarboxylase Broth (B.B.L.) was used with the addition of 1% (w/v) l-arginine hydrochloride (Edwards and Ewing 1962; Hendrie and Shewan 1966; Schroth and Hildebrand 1972). Indole production and the use of methyl-red as an indicator of final pH during glucose utilization were determined using Methyl red-Voges Proskauer medium (Difco). After three days cultures were tested with methyl red (Edwards & Ewing 1962).

For liquefaction of gelatin, cultures were inoculated into tubes containing Bacto Beef extract 3.0 g, Bacto peptone 5.0 g, Difco gelatin 120.0 g, distilled water 1,000 ml. Tubes were inoculated by deep stab and were incubated at room temperature and were checked at 3, 7, 14 and 21 days (Bailey and Scott 1974; Corpe 1953; Park and Holding 1966).

The oxidase reaction was determined by soaking a strip of filter paper with a few drops of p-aminodimethylalanine monohydrochloride (Difco) and immediately smearing a loopful of bacteria from a 24 hour agar culture on the moist paper. If a dark purple-black color appears, the organism is oxidase positive (Clarke and Richmond; 1975; Dowler and

Weaver 1975; Steel 1961).

Catalase production was determined by smearing a loopful of solid growth (24 hour P.C.A. culture) in a drop of 10 vol. hydrogen peroxide and examined for the evolution of gas bubbles (Bailey and Scott 1974). Urease slants (Difco) were inoculated and incubated at room temperature for 24 hours. The presence of a deep pink color, due to an increase in alkalinity, indicated the presence of urease (Edwards and Ewing 1962).

To test for starch hydrolysis, Yeast extract nutrient agar (yeast extract 5.0 g, bacto-peptone 5.0 g, bacto beef extract 5.0 g, distilled water 1000 ml, starch (Difco) 1% (w/v), pH 6.8) plates were spot-inoculated. After 48 to 96 hours incubation the plates were flooded with a dilute iodine solution. A clear halo around the colony indicates starch hydrolysis (Edwards and Ewing 1972; Bailey and Scott 1974).

Ability of organisms to denitrify was determined by growing them for 24 to 72 hours in tubes of Penassay Broth (Difco antibiotic medium #5) with two drops of 10% (w/v) KNO_3 added to each tube. Nitrate reduction was shown by a spot-test using diphenylamine and Trommsdorf reagent. Denitrification was shown by the absence of NO_3^- or NO_2^- in the medium after 24 hours of incubation (Dr. F.D. Cook, personal communication).

B. Pathogenicity

Because of the large numbers of isolates pathogenicity tests were conducted in two ways. Laboratory testing for pathogenicity to alfalfa seedlings and mature alfalfa root sections was done, in triplicate, on each isolate (Kreitlow 1963). Once the isolates have been identified to the genus and/or species level, representative organisms for each group

were tested for pathogenicity to alfalfa grown under greenhouse conditions.

For laboratory pathogenicity tests Beaver alfalfa seeds were used. The seeds were surface sterilized in 0.01% (w/v) $HgCl_2$ for fifteen minutes. This was followed by soaking in 1% (v/v) NaOCl for fifteen minutes, followed by a thorough rinsing with sterile distilled water. The seeds were transferred aseptically to petri plates containing modified Crones salts agar (Crones salts 1.0 g, glucose 0.5 g, distilled water 1000 ml) and were incubated in darkness for 72 to 96 hours at 25°C

After this time alfalfa seedlings showing no apparent contamination or discoloration of roots and having a root length of 1 to 2 cm long were used for stab inoculation. Roots were injured by puncturing the root near the tip with a sterile hypodermic needle (21G). Both wounded and unwounded alfalfa roots were inoculated with suspensions of bacterial isolates that were grown in T.S.B. for 48 to 56 hours and had an approximate cell concentration of 3×10^8 cells per ml. Sterile distilled water was used as a control. Inoculated plants were kept in petri plates containing modified Crones salts agar and were incubated at 25°C. After 72 to 96 hours the roots were examined for the development of necrosis and were rated using the disease index described previously.

In addition to seedling pathogenicity, sections of mature Beaver alfalfa roots were inoculated with the bacterial isolates and were examined for signs of necrosis. The mature alfalfa plants had been grown in sterile sand in the greenhouse for six months. Plants were harvested, roots washed free of sand and those roots free from discoloration and contamination were used. Primarily sections of the tap root and larger portions of lateral roots were used. These root

sections were surface-sterilized in 0.01% (w/v) $HgCl_2$ and 95% denatured ethanol as described previously. Two inch sections of root were aseptically excised and placed in petri plates containing water agar. Roots were wounded using a sterile hypodermic needle (21G). As before, wounded and unwounded roots were inoculated with bacterial suspensions grown for 72 hours in T.S.B. and had an approximate concentration of 3×10^8 cells/ml. Plates were incubated for three to five days at 25°C after which the roots were examined for signs of necrosis and rated for degree of lesioning.

C. Fungal Wounding of Roots

Once the identity of the isolates had been established the laboratory pathogenicity tests were repeated using representative organisms from each group plus a fungal isolate, *Fusarium* sp., provided by Dr. R. Reeleeder (MacDonald College). The bacterial isolates used were *B. polymyxa, B. subtilus, C. johnsonae, E. atroseptica, E. carotovora, Lysobacter enzymogenes, P. fluorescens,* and *P. marginalis*. Each isolate was tested for pathogenicity to alfalfa seedlings and mature alfalfa roots by itself and in combination with *Fusarium* sp. When the isolates were tested with *Fusarium*, the alfalfa roots were first inoculated with the fungal component and then twenty-four hours later with the bacterial component. This was done on the premise that the bacteria are opportunistic rather than invasive pathogens. After the inoculations the petri plates were incubated at 25°C. Sterile distilled water was used as the control inoculum. The plates were incubated for three to five days after which they were examined for the degree of lesioning.

D. Freeze Wounding of Roots

To test the feasibility of cracking due to alternate freezing and thawing acting as a mode of entry for phytopathogenic bacteria, segments of mature, healthy Beaver alfalfa roots were subjected to conditions of alternate freezing and thawing. The method used was primative but serves to illustrate the point. The roots were surface-sterilized prior to freezing and were then placed in sterile Whirlpack plastic bags (Canlab). The roots were kept moist to aid in freezing. They were kept at -10°C for two hours and then quickly immersed in a 35° - 40°C waterbath for ten minutes. This cycle of freezing and thawing was repeated five times. In the final step the roots were frozen overnight and next morning quickly thawed in the water bath. When examined the roots had a large number of brown, water-soaked areas which were the result of freezing. Two inch sections of root were excised and placed in petri plates containing water agar. Roots that had not been subjected to the freezing process were used as a control. The roots were then inoculated with bacterial suspensions that had grown in T.S.B. for 72 hours and had an approximate cell concentration of 1×10^8 cells/ml. The bacterial suspensions used were *B. polymyxa*, *B. subtilus*, *C. johnsonae*, *E. atroseptica*, *E. carotovora*, *Lysobacter enzymogenes*, *P. fluorescens* and *P. marginalis*. Sterile distilled water served as the control. All plates were incubated at 25°C for three to five days after which the degree of necrosis was determined.

E. Greenhouse Tests

Once the identity of the bacterial isolates had been established, a representative group of organisms were tested for pathogenicity to alfalfa seedlings. In initial experiments the Beaver alfalfa seedlings were in pots containing steam-sterilized soil and grown in a growth cabinet. Fifteen hours of light were provided and daytime temperatures ranged from 17°C to 21°C. The plants were fertilized twice weekly with 20-20-20.

Cultures of *E. atroseptica, E. carotovora, E. amylovora, Corynebacterium insidiosum, Pseudomonas corrugata, P. syringae, P. fluorescens* and *P. aeruginosa* were used. Sterile distilled water was the control. The organisms were grown on T.S.B. in a shaker culture. After four days the cells were harvested by centrifugation, washed three times in sterile physiological saline and resuspended in 5 ml of saline to an approximate concentration of 3×10^8 cells/ml. These solutions were used for inoculation. The alfalfa seeds were sterilized, germinated and wounded in the manner described previously. Twenty ml of inoculum were poured into a petri-dish and the wounded and unwounded alfalfa seedlings were allowed to soak in the inoculum for ten minutes. After this period the seedlings were planted in Hilson trays filled with sterilized greenhouse soil. Sixteen replicates of each treatement were used. In subsequent weeks the plants were rated for number of survivors, numbers of leaves at the various stages of growth and plant height. After four weeks the plants were harvested, their roots examined and rated for lesions and a mean fresh seedling weight calculated.

In the following experiments the concept that bacteria act together with other soil organisms, notably fungi, was tested. The fungus in question was a *Fusarium* sp. supplied by Dr. R. Reeleeder (McDonald College)

and had been isolated from lesioned alfalfa roots. The first experiment used a total of twenty treatments. These were divided into two major groups, wounded and unwounded alfalfa seedlings. Wounding was done as described previously and the alfalfa cultivar used was Beaver. Individual treatments consisted of each of the organisms alone. They were *E. atroseptica* (E-45), *L. enzymogenes* (MT82F-9), *C. johnsonae* (m12c-11), *P. fluorescens* (P-15), *Protrus vulgaris* (Department of Soil Science collection), *Rhizobium lipoferum* (Department of Soil Science collection) and *Azospirillum lipoferum* (Dr. F.V. MacHardy, Department of Soil Science, University of Alberta) and *Fusarium*species. *A lipoferum* and *R meliloti* were included as negative organism controls as neither were shown to cause lesioning or necrosis of alfalfa tissue by the methods described previously. The combined treatments consisted of each bacterial species in combination with *Fusarium* sp. The combined bacterial inoculations were *Erwinia + Lysobacter* and *Lysobacter + Cytophaga*.

Each Hilson tray held four treatments and there were ten trays in each replicate with a total of four replicates. During the growth period the plants were kept in a growth cabinet that provided fifteen hours of light each and a daytime temperature ranging between 17°C to 21°C. The plants were not given any additional fertilizer and were watered twice weekly with sterile distilled water. Plants were observed for number of survivors, plant height and the number of leaves at the different stages of growth. After four weeks the plants were harvested, roots washed free of soil and examined for degree of lesioning. A mean fresh seedlings weight was also calculated.

IV. RESULTS AND DISCUSSION

A. Isolation, Identification and Characterization of Isolates:

All the isolates came from plants showing typical alfalfa root rot symptoms. Fields exhibiting these symptoms were mainly grey Luvisols and had a gently undulating terrain. Depressional areas showed signs of periodic gleying with some mottling. Symptoms of alfalfa root rot were most striking in the depressions. Many fields had a severe weed problem with the most common weeds being brome grass (*Bromus inermis*), dandelion (*Taraxacum officinale*), stinkweed (*Polanisia graveolens*), Canada thistle (*Circium arvanse*), and foxtail (*Alapecurus pratensis*). Plate 1 shows one of the most dramatic fields that was sampled (Mallaig, Alberta). The large chlorotic areas in the background were slightly depressional, had a significant population of weeds, primarily dandelion, Canada thistle, foxtail and various grasses. These fields were sampled early in August 1980, two to three weeks after they had been cut for processing by the local dehydrating plant. Information was not available on the rate or type of fertilizer used on these fields. Generally after three to four years of continuous cropping with alfalfa, these fields are plowed under because of the significant decline in yield. They are then used for one to two years for/either barley or wheat production and then alfalfa is planted again. This would suggest that a certain population of pathogen is required and that the disease progresses slowly, in stages. Management practices are also an important factor to consider. The frequency with which the fields are cut may not allow the alfalfa plants to build up sufficient reserves to survive through the winter. Many fields in the Mallaig - St. Paul area and Mayerthorpe area received their last cut in the last week of September or early October. With the winters in

Plate 1

COLOURED PICTURES
Images en couleur

Field showing symptoms of "alfalfa sickness". Note chlorotic areas in background (Mallaig, Alberta).

Close-up of one of these chlorotic areas.

Plate 2

Close-up of an infected alfalfa stand near St. Paul, Alberta. Note the high population of weeds.

Overview of the above field. Stand was cut two weeks prior to Sampling.

COLOURED PICTURES
Images en couleur

north-central Alberta being quite severe and early, the plants may not have sufficient time to build up starch reserves for the winter. Hence a higher rate of winter-kill.

Another point to consider is that a great deal of alfalfa production is carried out on marginal land which may have a higher percentage of sloughs and is often gently undulating. An alfalfa stand growing in a depression is of a poorer quality than that growing at a slightly higher elevation. This is primarily due to waterlogging of the soil in the depressions. It is in these areas that symptoms of alfalfa root rot becomes most obvious.

Plate 3 illustrates typical crown lesions. The plants in the photograph were from a three year old stand of alfalfa. Note the extensive discoloration of the crown, the lesioning at the base of the lateral roots, the absence of fine roots and the absence of nodules. Plate 4 shown cross-sections of both the crown and lateral roots. The black area in the crown had lost the integrity of its original structure. Throughout the vascular tissues of the taproot and lateral roots were many tiny "salt and pepper"-like lesions. These lesions were not observed in plants less than one year old.

Isolations from lesions showed a very typical mixed flora of fungi and bacteria. The fungi were primarily *Fusarium* species and were identified microscopically on the presence or absence of septate hyphae and macroconidia. Chlamydospores of *Fusarium* were not observed. *Fusarium* appeared to be the predominant fungal species to be consistently isolated from lesions. *Phytophthora* species were not isolated by myself. Perhaps the fact that samples were collected in mid- to late summer may play a significant role. *Rhizopus* species were occasionally observed on some of the plates. Cereal

Plate 3

COLOURED PICTURES
Images en couleur

Crown lesions on a two year old alfalfa plant. Mayerthorpe, Alberta.

Close-up of same plant. Note absence of fine roots and the lesions on the lateral roots and tap root.

Plate 4.

COLOURED PICTURES
Images en couleur

Cross-section of a crown lesion. Note extensive necrosis of xylem.

Cross-section of a lateral root. Note necrosis and "salt and pepper" lesions throughout the root tissue

agar and V8 juice agar supported the highest population and variety of fungi while rose-bengal agar had the lowest. Plate count agar supported the greatest variety of bacteria. Bacteria also grew well on cereal agar, but intense competition by fungi on these media often made isolation of bacteria difficult.

Once the bacterial isolates were purified, they were screened for ability to macerate carrot and potato tissue. Isolates that did not pass this first screening were discarded and collectively grouped as saprophytes. Interestingly enough four isolates of a *Serratia* species, most likely *S. marcesens*, were able to initially macerate potato tissue but upon further cultivation in the laboratory, quickly lost this ability (Ewing 1962; Grimont *et. al.* 1979). Dr. F. L. Lukezic (personal communication, 1980) had isolated a large number of *S. marcescens* from discolored alfalfa roots and they produced a water-soluble, light-pink pigment on King's medium A. Initially these isolates were called *E. amylovora* var. *alfalfae*. This was based on their resemblance to *E. amylovora* in a number of physiological characters.

With two exceptions all isolates were motile, gram negative rods. The exceptions were isolates classified as *B. polymyxa* and *Clostridium* species. Pathogens such as *E. atroseptica, E. carotovora, P. fluorescens* were expected based on their well documented ability to cause root rot diseases (Burr and Schroth 1977; DeBoer *et. al.* 1978, 1979; Dye 1969a, b, c). The relative proportions of these pathogens was a bit surprising in that assumptions from information about the preference of *E. atroseptica* for cooler temperatures would lead one to expect that *E. atroseptica* would predominate in this climate. This was not the case. Roughly equal proportions of *E. atroseptica* and *E. carotovora* were found. However as

neither *E. atroseptica* or *E. carotovora* are host specific, it is not improbable to isolate both species from a single host. Seasonal distribution of these organisms may also be significant.

Fluorescent pseudomonads made up a significant portion of the pathogen population. A species of *Pseudomonas* has been described as the cause of a root rot of alfalfa in the U.S.S.R. (Rodighin and Petrov 1939). The host range of this pathogen, *Pseudomonas radiciperda,* encompassed *Trifolium pratense, Lens esculenta, Melilotus alba* and *Medicago sativa.* Due to the incomplete description of *P. radiciperda* and the lack of viable cultures, this organism has been dropped from the eighth edition of Bergy's Manual (Buchanan and Gibbons 1974).

Because of the minor differences in taxonomy between *P. fluorescens* and *P. marginalis, P. marginalis* is often grouped into *P. fluorescens* biotype II (Buchanan and Gibbons 1974). The major difference between these species is that isolates belonging to *P marginalis* are able to liquify pectate gels at pH 8.0, hence possessing an effective pectate lyase whereas those organisms grouped into *P. fluorescens* possessed only a polygalacturonase that is active at pH 5.0. Again relative proportions of both organism were roughly equal with neither species appearing to predominate in the root rot complex.

Alberta soils have been shown to have a wide spread population of *B. polymyxa* that were reported pathogenic to potato tubers (Jackson and Henry 1946). Other species of *Bacillus* reported to be pathogenic and pectinolytic are *Bacillus macerans, Bacillus licheniformis, Bacillus subtilus, Bacillus pantothenticus, Bacillus cereus, Bacillus circulans* and *Bacillus megaterium* (Ottow 1971). Only *B. polymyxa* was isolated from necrotic alfalfa. With the ability to sporulate under adverse

Table 3. Summary of physiological and biochemical characteristics of bacterial alfalfa root pathogens.

	Morphology				Physiology								Sugars									
					NaCl tolerance		O_2 requirement			Temperature °C												
	Shape	Gram	Motility	Flagella	5%	7%	Aerobic	Anaerobic	Spores	Min.	Max.	Opt.	Arabinose	Cellobiose	Fructose	Glucose	Lactose	Mannose	Maltose	Raffinose	Sucrose	Xylose
B. polymyxa	rod	+	+	Peri.	-	-	+	-	+	5	35-45	25	+		+	+	+	+	+	+	+	+
Chromobacterium sp.	rod	-	+	Polar	+	-	+	+	-	4	37	23	±	-	+	+	-	+	±	-	+	±
Clostridium sp.	rod	+	+	Peri.		-	-	+	+	4	37	25	-	+	+	+	+	+	+	+	+	+
Cytophaga johnsonae	rod	-	+	-	-	-	+	-	-	4	38	25	+	+	-	+	-	-	+	-	-	+
Erwinia atroseptica	rod	-	+	Peri.	+	-	+	+	-	3	37	23	+	+	+	+	+	+	+	+	+	+
Erwinia carotovora	rod	-	+	Peri.	+	+	+	+	-	6	37-40	25	+	+	+	+	+	±	±	+	+	+
Escherichia coli	rod	-	+	Peri.		-	+	+	-	5	25	37	+	-		+	+		+	+	+	+
Lysobacter sp.	rod	-	+	-	-	-	+	-	-	4	50	26	-	-	-	+	-	±	±	-	+	±
P. fluorescens	rod	-	+	Polar	+	-	+	-	-	3	40	25	+	±	+	+	-	+	+	±	-	+
P. marginalis	rod	-	+	Polar	+	±	+	-	-	4	34	23	+	±	+	+	+	+	+	+	+	-
P. syringae	rod	-	+	Polar	-	-	+	-	-	5	37	25	+		+	+		+			+	+
Serratia marcescens	rod	-	+	Peri.		-	+	+	-	6	38	25	-	+	±	+	-	-	+	-	+	-

Table 3. (cont'd)

	Alcohols		Polysaccharides		Miscellaneous biochemistry									Action on pectate gel					
	Ethanol	Mannitol	Cellulose	Pectin	Arginine Dihydrolase	Catalase	Citrate	Gelatin Liquefaction	Indole	M.R.	$NO_3 \rightarrow NO_2$	Oxidase	Pigments	Starch hydrolysis	Urease	V.P.	pH 5	pH 6.8	pH 8
B. polymyxa	-	+	-	+	-	+	±	+			+		none	+		+	+	+	-
Chromobacterium sp.	-	±	-	-	-	+	+	+	-	±	+	-	violaceaum	+	-	-	-	-	-
Clostridium sp.	-	-	+	+	-	-	-	-	-	-	-	-	none	+	-		-	+	-
Cytophaga johnsonae	-	-	+	+	-	+	-	+	-	-	+	-	bright orange yellow, pink	+	-	-	-	+	+
Erwinia atroseptica	-	+	-	+	-	+	+	+	-	-	+	-	none	-	-	-	-	+	-
Erwinia carotovora	+	+	-	+	-	+	+	+	+	-	+	-	none	-	-	-	-	+	-
Escherichia coli	-	+	-	-	-	+	-	-	+	+	+	-	none	-	-	-	-	-	-
Lysobacter sp.	-	-	+	+	-	+	-	+	-	-	+	+	yellow, brown pink, white	+	-	-	-	+	+
P. fluorescens	+	+	-	+	+	+	+	+	-		+	+	green fluorescent	-	+		-	+	-
P. marginalis	+	+	-	+	+	+	+	+	-		+	+	green fluorescent	-	+		-	-	+
P. syringae	-	+	-	+	-	+	-	+	-		-	-	green fluorescent	-	-		-	-	-
Serratia marcescens	-	+	-	-	-	+	+	+	+		+	-	pink, red	-	-	+	-	-	-

conditions compounded with its known role in soil mineral cycling, *B. polymyxa* is very well suited to persist in soil (Alexander 1977).

Clostridium species have been shown to produce large quantities pectic enzymes, a fact that is of major importance in the textile industry (Lee *et. al.* 1970; Lund and Brocklehurst 1978). This ability is used in the recovery of fibers such as flax and hemp (Lanigan 1959). Pectolytic clostridia have also been reported in association with other soft rot organisms in spoiled potato tissue (Lund 1972; Lund and Brocklehurst 1977). Lund (1979) reports the primary cause of the spoilage is *E. carotovora* and the pectolytic clostridia enhance the effect of *E. carotovora*. The pectolytic clostridia belong to several species but one group of clostridia forms a pink pigment and has many properties in common with *Clostridium felsineum* (Lund 1979). The pectolytic clostridia isolated from alfalfa lesions were not classified to the species level primarily due to the difficult and fastidious nature of the isolates.

Organisms such as *B. polymyxa* and *Clostridium* may often be overlooked when isolating from diseased material. Both organisms appeared to predominate on B10 agar and produced distinctive colonies. *B. polymyxa* colonies produced a small pit around themselves. This pit was not due to liquefaction of the agar. Colonies of *Clostridium* produced blackening, due to sulfite production, around colonies after seven days incubation. Both these organisms were non-descript on any of the other media used.

Both *Chromobacterium* species and *Serratia* species produced their characteristic pigments upon initial isolation. This character was lost upon subsequent cultivation. As neither of these organisms is cellulolytic or strongly pectinolytic, their role in a root disease complex is a puzzle. Both *Chromobacterium* and *Serratia* are strongly

Table 4. Pathogenicity of isolates

	Ability to rot			Ability to cause lesions on			
	Carrots	Onions	Potatoes	Alfalfa seedling	Disease index	Mature alfalfa roots	Disease index
B. polymyxa							
E-11	+	+	+	+	3	+	2+
M12F-4	+	+	+	+	3	+	2+
M12F-7	+	+	+	+	3	+	2+
M12F-11	+	+	+	+	4	+	2
M12P-4	+	+	+	+	3	+	2
MT12P-3	+	+	+	+	4	+	2
MT14P-9	+	+	+	+	3	+	2
MT21F-4	+	+	+	+	3	+	2
MT11P-5	+	+	+	+	3+	+	2
MT11P-6	+	+	+	+	2	+	2
MT12P-2	+	+	+	+	3	+	2
MT12P-5	+	+	+	+	4	+	3
MT12F-4	+	+	+	+	4	+	2
MT23F-6	+	+	+	+	3+	+	2
SP1-1	+	+	+	+	2	-	1
SP1-4	+	+	+	+	2	-	1
Clostridium sp.							
M12C-10	+	+	+	+	3	+	3
MT12P-2	+	+	+	+	3	+	2+
MT14P-3	+	+	+	+	4	+	2
MT11P-9	+	+	+	+	3+	+	2+
SP3-1	+	+	+	+	3+	+	3
SP4	+	+	+	+	3	+	2
Chromobacterium violaceum							
E-33	+	+	+	+	2	-	1
E-53	+	+	+	+	2	-	1
M12F-12	+	+	+	+	2	-	1
MT14F-8	+	+	+	+	2	0	1
MT14F-4	+	+	+	+	2	-	1
MT14F-6	+	+	+	+	2	-	1
MT11F-8	+	+	+	+	2	-	1
MT14F-6	+	+	+	+	3	+	2
MT82F-5	+	+	+	+	2	-	1
Cytophaga johnsonae							
P-17	+	+	+	+	3	-	1
E-3	+	+	+	+	3	-	1
E-40	+	+	+	+	3	-	1
E-41	+	+	+	+	2+	-	1
M11C-2	+	+	+	+	2+	-	1
M12F-1	+	+	+	+	2+	+	2
M12F-2	+	+	+	+	3	-	1
M12F-8	+	+	+	+	3	-	1
M12C-2	+	+	+	+	3	-	1
M12C-8	+	+	+	+	3	-	1
M12C-11	+	+	+	+	2+	-	1
M12C-14	+	+	+	+	2+	-	1
M12P-6	+	+	+	+	2	±	2
M12P-9	+	+	+	+	2	-	1
MT11P-2	+	+	+	+	2	+	2
MT11P-3	+	+	+	+	2+	+	2
MT11P-5	+	+	+	+	2+	+	2
MT11P-6	+	+	+	+	3	+	2
MT11P-7	+	+	+	+	3	-	1
MT11P-8	+	+	+	+	3	-	1
MT11F-8	+	+	+	+	3	-	1

Table 4. Pathogenicity of isolates (cont'd)

	Ability to rot			Ability to cause lesions on			
	Carrots	Onions	Potatoes	Alfalfa seedlings	Disease index	Mature alfalfa roots	Disease index
Cytophaga johnsonae							
MT12F-1	+	+	+	+	2+	-	1
MT12F-2	+	+	+	+	2	-	1
MT12F-3	+	+	+	+	2	-	1
MT12F-4	+	+	+	+	2+	-	1
MT12F-5	+	+	+	+	2+	-	1
MT12F-6	+	+	+	+	3	-	1
MT12F-7	+	+	+	+	3	-	1
MT12F-8	+	+	+	+	2+	-	1
MT12P-3	+	+	+	+	2+	-	1
MT12P-4	+	+	+	+	2+	-	1
MT14P-1	+	+	+	+	2+	+	2
MT21C-1	+	+	+	+	2+	+	2
MT21C-2	+	+	+	+	2+	+	2
MT21F-1	+	+	+	+	2	-	1
MT21F-2	+	+	+	+	2+	-	1
MT21P-3	+	+	+	+	2+	-	1
MT21P-7	+	+	+	+	2+	-	1
MT21P-5	+	+	+	+	2	-	1
MT21P-6	+	+	+	+	2	-	1
MT23F-2	+	+	+	+	2	-	1
MT23P-1	+	+	+	+	2	-	1
MT23P-2	+	+	+	+	2	-	1
MT23P-4	+	+	+	+	2	-	1
MT92P-1	+	+	+	+	2	-	1
MT92P-2	+	+	+	+	2	-	1
MT82P-3	+	+	+	+	2+	-	1
MT82P-8	+	+	+	+	2+	-	1
MT23C-1	+	+	+	+	3	-	1
MT23C-2	+	+	+	+	2+	-	1
MT23C-3	+	+	+	+	2+	-	1
MT23C-4	+	+	+	+	2+	-	1
MT84P-5	+	+	+	+	2+	-	1
MT84P-6	+	+	+	+	2+	-	1
MT84P-1	+	+	+	+	2+	-	1
SP5-2	+	+	+	+	2+	-	1
E. atroseptica							
P-14	+	+	+	+	4	+	3
P-20	+	+	+	+	4	+	3
P-27	+	+	+	+	5	+	4
P-30	+	+	+	+	5	+	5
E-2	+	+	+	+	5	+	4
E-4	+	+	+	+	4	+	4
E-7	+	+	+	+	5	+	4
E-12	+	+	+	+	4+	+	3
E-17	+	+	+	+	4	+	3
E-45	+	+	+	+	4	+	3+
E-47	+	+	+	+	4	+	3
E-54	+	+	+	+	4	+	3
E-58	+	+	+	+	5	+	3
M12F-10	+	+	+	+	5	+	2+
M12-C	+	+	+	+	5	+	3
M12C-6	+	+	+	+	4	+	2
M12C-7	+	+	+	+	4	-	1
M12C-12	+	+	+	+	4	-	1
M12C-13	+	+	+	+	4	-	1
MT21P-1	+	+	+	+	4	+	3
MT21P-7	+	+	+	+	4	+	2+
MT11F-9	+	+	+	+	4	+	2
SP-2	+	+	+	+	5	+	4
SP-3	+	+	+	+	4	+	3

Table 4. Pathogenicity of isolates (cont'd)

	Ability to rot			Albilty to cause lesions on			
	Carrots	Onions	Potatoes	Alfalfa seedling	Disease index	Mature alfalfa roots	Disease index
E. carotovora							
E-1	+	+	+	+	$	+	#
E-10	+	+	+	+	4	+	3
E-13	+	+	+	+	4	+	3
E-18	+	+	+	+	4	+	3
E-29	+	+	+	+	4	+	3
E-32	+	+	+	+	5	+	3
E-34	+	+	+	+	5	+	3
E-35	+	+	+	+	3	-	1
E-36	+	+	+	+	5	+	2+
E-43	+	+	+	+	5	+	4
E-44	+	+	+	+	4	+	3
E-46	+	+	+	+	4	+	3
E-48	+	+	+	+	4	+	2+
E-50	+	+	+	+	5	+	4
E-51	+	+	+	+	5	+	2+
E-56	+	+	+	+	4	+	2+
M12F-5	+	+	+	+	4	+	3
M12F-6	+	+	+	+	4	+	3
M12F-9	+	+	+	+	4	+	2+
M12F-13	+	+	+	+	4	-	1
M12P-2	+	+	+	+	4	-	1
M12P-5	+	+	+	+	4	+	3
M12P-7	+	+	+	+	4	+	3
M12P-8	+	+	+	+	4	+	2+
M21C-3	+	+	+	+	4	+	2+
SP1-1	+	+	+	+	5	+	3
SP6-2	+	+	+	+	4	+	2+
SP6-6	+	+	+	+	4	+	2
Lysobacter enzymogenes							
E-37	+	+	+	+	2	+	2
E-39	+	+	+	+	2	+	2
M12C-1	+	+	+	+	2+	+	2
MT82F-9	+	+	+	+	2+	+	2
MT82F-10	+	+	+	+	2+	+	2
MT23F-1	+	+	+	+	2+	+	2
MT23F-5	+	+	+	+	2+	+	2
MT23F-8	+	+	+	+	2	-	1
MT23F-9	+	+	+	+	2	-	1
MT23F-11	+	+	+	+	2	+	2
SP1-9	+	+	+	+	2	-	1
SP6-3	+	+	+	+	2	-	1
SP7-1	+	+	+	+	2	-	1
P. fluorescens							
P-1	+	+	+	+	2+	-	1
P-2	+	+	+	+	2+	-	1
P-3	+	+	+	+	3	+	2
P-4	+	+	+	+	3	+	2
P-8	+	+	+	+	3	+	2
P-9	+	+	+	+	3	+	2
P-11	+	+	+	+	3	+	2
P-13	+	+	+	+	2	-	1
P-15	+	+	+	+	2	-	1
P-18	+	+	+	+	2	-	1
P-19	+	+	+	+	2+	+	2
P-22	+	+	+	+	2	-	1
P-26	+	+	+	+	2	-	1
P-29	+	+	+	+	3	+	2
P-31	+	+	+	+	3	-	1
P-32	+	+	+	+	2+	-	1
P-33	+	+	+	+	2+	-	1
P-34	+	+	+	+	3	+	2

Table 4. Pathogenicity of isolates (cont'd)

	Ability to rot			Ability to cause lesions on			
	Carrots	Onions	Potatoes	Alfalfa seedling	Disease index	Mature alfalfa roots	Disease index
P. fluorescens							
E-16	+	+	+	+	2+	-	1
E-23	+	+	+	+	3	-	1
E-38	+	+	+	+	2	-	1
E-42	+	+	+	+	2	-	1
E-52	+	+	+	+	3	-	1
M12C-3	+	+	+	+	3	+	2
M12C-4	+	+	+	+	3	+	2
M12C-9	+	+	+	+	3	+	2
M12P-1	+	+	+	+	3	+	2
M11C-3	+	+	+	+	3	+	2
M11C-4	+	+	+	+	2+	+	2
M11C-5	+	+	+	+	2+	+	2
MT11F-1	+	+	+	+	2	-	1
MT11F-2	+	+	+	+	3	+	2
MT11F-3	+	+	+	+	3	+	2
MT11F-4	+	+	+	+	3	+	2
MT11F-5	+	+	+	+	3	+	2
MT11F-6	+	+	+	+	2+	-	1
MT11F-7	+	+	+	+	2+	-	1
MT11F-10	+	+	+	+	2+	-	1
MT11P-1	+	+	+	+	3+	+	2
MT11P-2	+	+	+	+	3	+	2
MT11P-4	+	+	+	+	2	+	2
MT11P-7	+	+	+	+	3	-	1
MT12P-3	+	+	+	+	2+	-	1
MT14F-1	+	+	+	+	3	+	2
MT14F-2	+	+	+	+	4	+	2
MT14F-3	+	+	+	+	2	-	1
MT14P-1	+	+	+	+	2	-	1
MT14P-2	+	+	+	+	3	-	1
MT14P-4	+	+	+	+	2	-	1
MT14P-5	+	+	+	+	2	-	1
MT14P-6	+	+	+	+	3	-	1
MT14P-7	+	+	+	+	3	-	1
MT14P-8	+	+	+	+	2	-	1
MT14P-9	+	+	+	+	3	-	1
MT14P-10	+	+	+	+	3	-	1
MT14P-11	+	+	+	+	3	-	1
MT14P-12	+	+	+	+	2	-	1
MT21C-4	+	+	+	+	2	-	1
MT21C-5	+	+	+	+	2	+	2
MT21P-2	+	+	+	+	3	+	2
MT21P-3	+	+	+	+	3	+	2
MT21P-4	+	+	+	+	3	+	2
MT21F-5	+	+	+	+	4	-	1
MT23F-2	+	+	+	+	2	-	1
MT23F-3	+	+	+	+	2	+	2
MT23F-4	+	+	+	+	2+	-	1
MT82F-4	+	+	+	+	3+	+	2
MT82F-6	+	+	+	+	3+	+	2
MT82F-7	+	+	+	+	4	+	2
MT84F-1	+	+	+	+	2	-	1
MT84F-2	+	+	+	+	2	-	1
MT84F-3	+	+	+	+	2	-	1
MT84F-4	+	+	+	+	2	-	1
MT84P-2	+	+	+	+	2	-	1
MT84P-3	+	+	+	+	2	-	1
SP1-13	+	+	+	+	2	-	1
SP1-5	+	+	+	+	2	-	1
SP1-6	+	+	+	+	2	-	1
SP1-8	+	+	+	+	2	-	1
SP6-4	+	+	+	+	3	-	1
SP6-5	+	+	+	+	3	+	2

Table 4. Pathogenicity of isolates (cont'd)

	Ability to rot			Ability to cause lesions on			
	Carrots	Onions	Potatoes	Alfalfa seedling	Disease index	Mature alfafla roots	Disease index
P. marginalis							
P-12	+	+	+	+	4	+	2
P-21	+	+	+	+	3	-	1
P-35	+	+	+	+	3	-	1
E-19	+	+	+	+	3	+	2
E-20	+	+	+	+	5	+	2
E-40	+	+	+	+	4	+	2
E-57	+	+	+	+	4	+	2
M12F-14	+	+	+	+	3	-	1
M11C-7	+	+	+	+	3	-	1
MT11P-8	+	+	+	+	4	+	2
MT14F-5	+	+	+	+	4	+	2
MT11P-10	+	+	+	+	4	-	1
MT14P-10	+	+	+	+	4	-	1
MT21C-3	+	+	+	+	3	+	2
MT21P-1	+	+	+	+	3	+	2
MT21F-6	+	+	+	+	4	+	2
MT23P-3	+	+	+	+	4	-	1
MT23C-5	+	+	+	+	4	-	1
Serratia sp.							
P-28	-	-	±	±	1+	-	1
MT11P-9	-	-	-	-	1	-	1
MT11F-9	-	-	-	-	1	-	1
MT12P-1	-	-	-	-	1	-	1

saccharolytic whereas the fluorescent pseudomonads and soft-rot *Erwinia* are not. It is possible the *Serratia* and *Chromobacterium* may function as tertiary pathogens. They are not directly involved in the maceration process but are able to complete the destruction wrought by pectinolytic bacteria by consuming the plant's starch reserves. It is not inconceivable to think of other so-called saprophytic bacteria found in the lesions to have a similar function.

Both *Cytophaga* species and *Lysobacter* species are known to be strongly cellulolytic, pectinolytic, proteolytic and saccharolytic (Christensen 1977; Christensen and Cook 1978). In short these two genera could be the ideal pathogen, fully equiped to utilize all plant components. A significant portion of the alfalfa isolates belonged to these two genera. Considering the capabilities these organisms have at their disposal, it is remarkable not to find more reports of them in the literature as significant plant pathogens.

Plates 5 through 10 show the major pathogens found on eigher sterile onion, potato or carrot tissue. With pathogens such as *B. polymyxa* and *E. carotovora,* maceration of tissue was accompanied by oxidation of tannins in the vegetable tissue, hence the black discoloration. In all cases maceration proceed quickly under room temperature. Potato tissue consistently had the greatest degree of tissue destruction while onion tissue had the least. On plate 7 where *B. polymyxa + E. atroseptica + Cytophaga + P. fluorescens* were inoculated onto potato tissue, the degree of necrosis is not as great as one would expect given the individual reactions of these organisms. As Cytophaga species have been shown to lyse a number of microorganisms, including gram-negative and gram-positive bacteria, blue-green bacteria, fungi, green algae and nematodes

Plate 5

COLOURED PICTURES
Images en couleur

Uninoculated potato control.

P. fluorescens on wounded potato.

Plate 6.

COLOURED PICTURES
Images en couleur

E. atroseptica on wounded potato.

Cytophaga on wounded potato.

Plate 7.

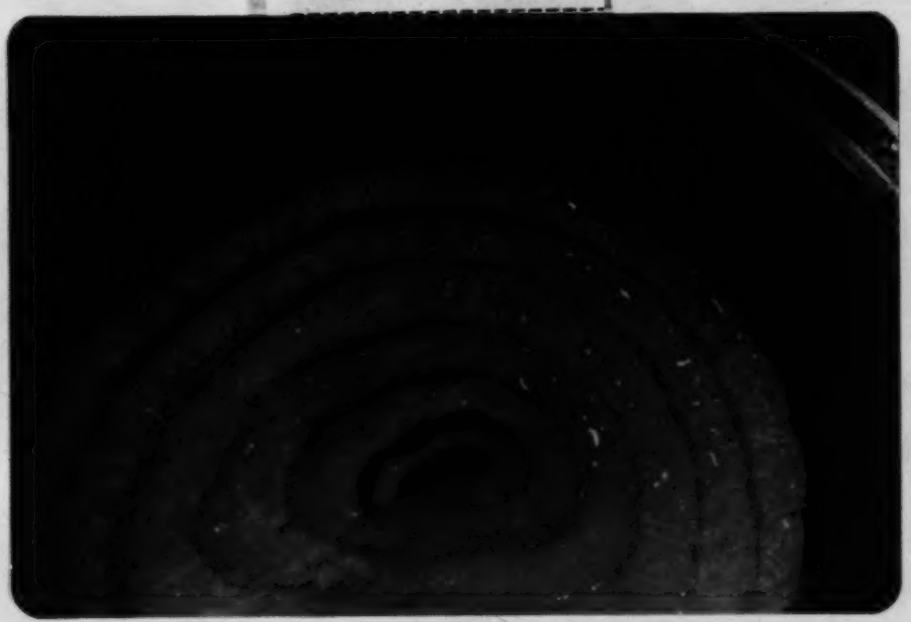
COLOURED PICTURES
Images en couleur

B. polymyxa on wounded potato.

B. polymyxa + *E. atroseptica* + *Cytophaga* + *P. fluorescens* on wounded potato.

Plate 8.

COLOURED PICTURES
Images en couleur



Uninoculated onion control.

P. fluorescens on wounded onion.

Plate 9.

COLOURED PICTURES
Images en couleur

E. atroseptica on wounded onion.

Uninoculated carrot control.

Plate 10.

COLOURED PICTURES
Images en couleur

P. fluorescens on wounded carrot.

Lysobacter on wounded carrot.

(Christensen, 1973) it would appear that in addition to attacking plant tissues, the *Cytophaga* may also be consuming its fellow pathogens. This could explain why the destruction of tissue is not as complete as expected.

B. Wounding

All of the isolates appear to require some type of wound in order to initiate successful infection. Lesioning, as seen in table 3, appears to be most severe in alfalfa seedlings. This is due to the lack of materials such as lignins, which afford the plant a certain degree of protection. It is for this reason that lesion rating on segments of mature alfalfa root is not as severe as that observed on alfalfa seedlings. Of all the isolates, *E. atroseptica* and *E. carotovora* cause the most severe lesioning on mature alfalfa roots while *Cytophaga*, *Lysobacter* and *Pseudomonas* cause the least severe. This could be due to the type and amount of pectic enzymes produced. As mentioned previously, *Erwinia* species have an inducible synthesis of pectate lyase, while pseudomonads have a constitutive synthesis (Zucker *et. al.* 1972). Thus when *Erwinia* is confronted with a source of pectate, it is able to produce more pectate lyase more quickly than a pseudomonad. Judging from the results, *Cytophaga* and *Lysobacter* may be subject to catabolite repression by a mechanism similar to that expressed by *Pseudomonas*.

C. Fungal Wounding

Fungal wounding of roots was an attempt to simulate a more natural type of wounding. With the combination of *Fusarium* plus bacteria three outcomes were possible. These were, plants would show no change over individual effects of the organisms used, there would be a decrease in disease symptoms or there would be an increase in disease symptoms. As

Plate 11

COLOURED PICTURES
Images en couleur

Control. Unwounded seedling on the left and a wounded seedling on the right.

Fusarium species on an unwounded alfalfa seedling (bottom) and a wounded alfalfa seedling (top)

Plate 12.

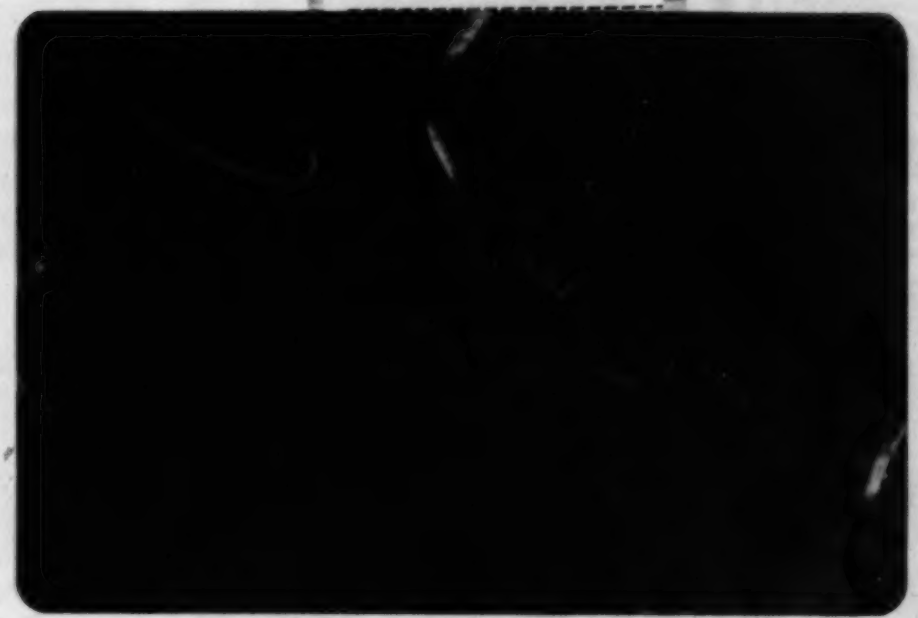
COLOURED PICTURES
Images en couleur

C. johnsonae on a wounded alfalfa seedling.

E. atroseptica on a wounded alfalfa seedling.

Plate 13

COLOURED PICTURES
Images en couleur



P. fluorescens on a wounded alfalfa seedling.

B. polymyxa on a wounded rapeseed seedling. Note the girdling in the lower half of the root.

Table 5. Fungal wounding of alfalfa roots

	Ability to cause necrosis on			
	Alfalfa seedlings	Disease index	Mature alfalfa root sections	Disease index
Control	-	1	-	1
Fusarium	+	4	+	2
B. polymyxa	+	2	+	2
B. subtilus	-	1	-	1
C. johnsonae	+	2	-	1
E. atroseptica	+	4	+	2
E. carotovora	+	4	+	2
Lysobacter	+	2	-	1
P. fluorescens	+	2	+	2
P. marginalis	+	3	+	2
Fusarium + *B. polymyxa*	+	4	+	4
Fusarium + *B subtilus*	+	3	+	3
Fusarium + *C. johnsonae*	+	5	+	3
Fusarium + *E atroseptica*	+	5	+	3
Fusarium + *E carotovora*	+	5	+	4
Fusarium + *Lysobacter*	+	4	+	3
Fusarium + *P. fluorescens*	+	5	+	3
Fusarium + *P. marginalis*	+	5	+	3

with mechanical wounding experiments, sections of mature alfalfa roots showed less severe lesioning that did alfalfa seedlings. This again was a function of complexity of root structure. With the exception of *Fusarium + Lysobacter*, fungal wounding generally produced a more severe lesion that either component alone. Roots showed a greater degree of discoloration and girdling. Enhancement of symptoms was generally observed when combinations of pathogens were inoculated. The exception to this was the combination of *Fusarium* and *Lysobacter*. Disease symptoms appeared less severe than that exhibited by *Fusarium* alone. With the known affinity for *Lysobacter* for fungi as a food source, it is not surprising to postulate that the *Fusarium* may be acting as a second substrate. When confronted with two equally attractive substrates, the *Lysobacter* may very well be partaking of both.

D. Freeze-wounding

The attempt at freeze-wounding of mature alfalfa roots was not as successful as hoped for. This was due to the primative nature of the method used. However the results do indicate that alternate freezing and thawing are important factors to be considered when discussing avenues of entry for opportunistic invaders. For the more strongly pectinolytic bacteria such as *B. polymyxa, E. atroseptica, P. fluorescens* and *P. marginalis*, these wounds that were created were sufficient to allow entry of the pathogen. The frozen control root was more discolored than the unfrozen control. Necrosis in the frozen roots was darker in color than that observed in unfrozen roots. However in the cooler climate in northern Alberta, this is likely an important avenue of plant root wounding. This is especially significant in depressional areas, showing signs of seasonal gleying, such as those areas where the greatest

Table 6. Freeze-wounding of alfalfa roots.

	Necrosis	Disease index
A. Conventional wounding		
Control	-	1
B. polymyxa	+	2
B. subtilus	-	1
C. johnsonae	+	2
E. atroseptica	+	3
E. carotovora	+	3
Lysobacter enzymogenes	+	2
P. fluorescens	+	2
P. marginalis	+	2
B. Freeze-wounding		
Control	-	1
B. polymyxa	+	2
B. subtilus	-	1
C. johnsonae	-	1
E. atroseptica	+	2
E. carotovora	+	2
Lysobacter enzymogenes	-	1
P. fluorescens	+	2
P. marginalis	+	2

incidence of alfalfa sickness symptoms are observed.

E. Greenhouse experiments.

In the first greenhouse experiment using only bacterial pathogens, the general trend appeared to follow that set in the laboratory. Pathogens such as *Erwinia* and *Pseudomonas* had the greatest effect on seedling growth. Seedlings treated with *Erwinia* that succumb to root rot, showed extensive discoloration in the root and in the stem. These plants were girdled at the soil-air interface. These symptoms are similar to those described for damping-off by fungi such as *Phytophthora* species (Agrios 1978).

Wounded seedlings showed more severe disease symptoms than did unwounded. This too was expected as the bacterial pathogens are opportunistic. Organisms such as *C. insidiosum, E. amylovora, P. aeruginosa* and *P. syringae* did not appear to restrict plant growth too severly and did not cause any significant degree of lesioning to the roots. *P. syringae* has been reported to be involved in other necrotic disease complexes, blackspot of rapeseed pods (Dr. A.W. Henry, University of Alberta, personal communication), so one might have expected to observe a similar effect on alfalfa.

As infection in the plant progressed, the seedlings began to show physical symptoms of stress, such as stunting and chlorosis of leaves. Leaves in these experiments actually refers to the number of leaflets the plant has produced. Chlorosis was not evident until the third week of growth. Because these plants were fertilized regularily with 20-20-20, it can be assumed that chlorosis is not due to nutrient deficiencies, but rather a function of infection. Wounded seedlings showed more severe disease symptoms than unwounded. This too was

Table 7. Effect of selected bacterial pathogens on the growth of wounded and unwounded alfalfa plants

Treatment	1 Week			2 Weeks			3 Weeks			4 Weeks		
	Number of survivors ($\bar{x}$/16) ($S=\pm1$)	Number of leaves ($\bar{y}$) ($S=\pm1$)	Mean plant height (cm) ($S=\pm1$)	Number of survivors (x/16) ($S=\pm1$)	Number of leaves ($\bar{y}$) ($S=\pm1$)	Mean plant height (cm) ($S\bar{x}=\pm0.025$)	Number of survivors ($\bar{x}$/16) ($S=\pm1$)	Number of leaves ($\bar{y}$) ($S=\pm1$)	Mean plant height (cm) ($S=\pm0.1$)	Number of survivors ($\bar{x}$/16) ($S=\pm1$)	Number of leaves ($\bar{y}$) ($S=\pm1$)	Mean plant height (cm) ($S\bar{x}=\pm0.025$)
UNWOUNDED												
Control	16	2	1.5	16	3	2.1	16	4	3.2	16	10	5.5
C. insidiosum	16	2	1.3	16	3	1.9	16	4	2.8	16	8	4.9
E. amylovora	16	2	1.5	16	3	2.0	16	4	2.9	15	10	5.2
E. atroseptica	14	2	1.0	14	2	1.9	13	3*	2.5	13	5*	4.0
E. carotovora	16	2	1.2	16	2	1.6	16	4*	2.7	15	7*	3.8
P. aeruginosa	16	2	1.4	16	3	1.9	16	4	3.1	16	9	4.2
P. corrugata	13	2	0.9	13	3	1.6	13	4*	2.8	13	7*	4.9
P. fluorescens	15	2	1.1	15	2	1.5	14	4*	2.6	13	8*	4.3
P. syringae	16	2	1.3	16	3	1.8	16	5	2.9	16	8	5.0
WOUNDED												
Control	16	3	1.3	15	3	1.9	16	4	3.0	16	11	5.3
C. insidiosum	16	2	1.0	16	3	1.9	16	4	3.0	16	8	5.0
E. amylovora	16	2	1.0	16	3	2.0	16	5	2.8	16	8	5.2
E. atroseptica	13	2	0.8	12	2	1.5	9	4*	2.3	9	8*	4.5
E. carotovora	15	2	0.8	15	2	1.7	14	4*	2.0	13	8*	3.5
P. aeruginosa	16	2	1.2	16	3	2.0	16	5	2.5	16	9*	4.7
P. corrugata	15	2	1.1	15	2	1.6	14	4*	2.0	14	10*	4.5
P. fluorescens	14	2	1.0	14	2	1.6	14	4*	2.2	14	9*	4.5
P. syringae	16	2	1.2	16	2	1.9	16	4	2.8	16	11	4.8

* chlorotic.

Table 8. Effect of selected bacterial pathogens on disease index and yield of alfalfa grown in the greenhouse

Treatment	Disease index	Mean fresh seedling weight (g) (Sx=±0.003)
UNWOUNDED		
Control	1	0.285
C. insidiosum	1	0.261
E. amylovora	1	0.273
E. atroseptica	3	0.239
E. carotovora	3	0.230
P. aeruginosa	1	0.252
P. corrugata	2	0.260
P. fluorescens	2	0.257
P. syringae	1	0.267
WOUNDED		
Control	1	0.273
C. insidiosum	1	0.268
E. amylovora	1	0.271
E. atroseptica	4	0.254
E. carotovora	4	0.227
P. aeruginosa	1	0.256
P. corrugata	3	0.256
P. fluorescens	2	0.231
P. syringae	1	0.259

expected as the bacterial pathogens are opportunistic.

Overall *P. fluorescens* and *E. carotovora* had the greatest effect on mean seedling weight. In the case of plants inoculated with *P. fluorescens*, lesion rating was not as severe as that observed with some of the other pathogens, however, the plants exhibited more severe chlorosis and stunting than in other trials. Two explanations are possible, the first is that the physical presence of these organisms in the xylem would cause a partial blockage restricting the upward flow of nutrients and water. This is a gradual process so the effect on the seedlings would require a certain length of time to manifest symptoms. However this type of mechanism would be operating with all the other bacterial pathogens where the physical presence of bacteria in the xylem impede the upward translocation of water and nutrients.

The second explanation involves the pathogenic capabilities of *P. fluorescens* and the regulatory mechanisms involved. Although *P. fluorescens* has been shown to produce moderate to large quantities of extracellular pectate lyases, synthesis of these enzymes appears to be subject to some form of catabolite repression (Codner, 1971). Prior to inoculation into the seedlings, the pathogens were grown on a glucose-rich medium. Work carried out by Zucker *et. al.* (1972) demonstrates that synthesis of pectate lyases is enhanced when fluorescent pseudomonads are grown in media with a vegetable extract added.

In the second set of greenhouse experiments involving phytopathogenic bacteria in combination with *Fusarium* and each other, results seem to indicate that the combined activity of most pathogens enhances the effect on the host. In experiments involving more than one pathogen it is also

Table 9. Effect of combined action of bacterial pathogens + *Fusarium* on the growth of wounded and unwounded alfalfa seedlings grown in the greenhouse.

Treatment	1 Week			2 Weeks			3 Weeks			4 Weeks		
	Number of survivors ($\bar{x}$/16) (S=±1)	Number of leaves ($\bar{y}$) (S=±1)	Mean plant height (cm) (S=±0.1)	Number of survivors ($\bar{x}$/16) (S=±1)	Number of leaves ($\bar{y}$) (S=±1)	Mean plant height (cm) (S=±0.05)	Number of survivors ($\bar{x}$/16) (S=±1)	Number of leaves ($\bar{y}$) (S=±1)	Mean plant height (cm) (S=±0.1)	Number of survivors ($\bar{x}$/16) (S=±1)	Number of leaves ($\bar{y}$) (S=±1)	Mean plant height (cm) (S=±0.05)
UNWOUNDED												
Control	16	2	1.8	16	3	2.5	16	5	3.9	16	12	5.8
E. atroseptica	15	2	1.4	14	2	1.9	14	4*	2.5	14	8*	3.9
C. johnsonae	15	2	1.6	15	4	2.2	15	4	3.2	15	8*	4.3
Lysobacter sp.	16	2	1.4	16	4	2.0	16	5	3.0	16	10	3.9
P. fluorescens	16	2	1.3	16	3	2.0	16	5*	2.8	16	10*	4.2
P. vulgaris	16	2	1.5	16	3	2.3	16	6	3.3	16	10	4.7
R. meliloti	16	2	1.8	16	4	2.7	16	6	4.1	16	12	6.0
S. lipoferum	16	2	1.7	16	3	2.5	16	5	4.0	16	10	5.6
Fusarium	14	2	1.3	13	2	1.8	12	4*	2.6	12	8*	3.5
E. atroseptica + *Fusarium*	14	2	1.4	13	2	1.9	12	4*	2.6	11	8*	3.3
C. johnsonae + *Fusarium*	15	2	1.6	15	3	2.0	15	4*	2.9	15	10*	3.5
Lysobacter + *Fusarium*	15	2	1.5	15	3	1.9	15	4*	2.7	15	8*	4.0
P. fluorescens + *Fusarium*	15	2	1.3	15	4	1.8	15	4*	2.5	14	10*	4.2
P. vulgaris + *Fusarium*	15	2	1.3	15	4	1.7	15	5*	2.9	15	10	4.8
R. meliloti + *Fusarium*	15	2	1.6	15	3	2.1	15	5*	2.9	15	12	6.0
S. lipoferum + *Fusarium*	15	2	1.6	15	4	2.0	15	4*	2.9	15	10	5.7
E. atroseptica + *Lysobacter*	13	2	1.4	13	3	1.7	13	5*	2.6	12	9*	3.6
Lysobacter + *Cytophaga*	15	2	1.6	15	3	2.0	15	5*	3.0	15	10*	3.8
E. atroseptica + *Lysobacter* + *Fusarium*	14	2	1.2	13	2	1.6	10	4*	2.2	10	8*	3.2
Lysobacter + *Cytophaga* + *Fusarium*	15	2	1.3	14	2	1.6	10	4*	2.0	10	10*	3.4

Table 9. Effect of combined action of bacterial pathogens + *Fusarium* on the growth of wounded and unwounded alfalfa seedlings grown in the greenhouse (cont'd)

Treatment	1 Week			2 Weeks			3 Weeks			4 Weeks		
	Number of survivors ($\bar{x}$/16) (S-±1)	Number of leaves ($\bar{y}$) (S-±1)	Mean plant height (cm) (S-±0.1)	Number of survivors ($\bar{x}$/16) (S-±1)	Number of leaves ($\bar{y}$) (S-±1)	Mean plant height (cm) (S-±0.05)	Number of survivors ($\bar{x}$/16) (S-±1)	Number of leaves ($\bar{y}$) (S-±1)	Mean plant height (cm) (S-±0.1)	Number of survivors ($\bar{x}$/16) (S-±1)	Number of leaves ($\bar{y}$) (S-±1)	Mean plant height (cm) ($S\bar{x}$-±0.05)
WOUNDED												
Control	16	2	1.6	16	4	2.5	16	6	3.8	16	12	5.5
E. atroseptica	14	2	1.2	14	4	1.9	11	6*	2.8	10	9*	4.9
C. johnsonae	16	2	1.4	16	3	2.2	11	6*	3.2	15	10*	4.3
Lysobacter	16	2	1.5	16	3	2.0	16	6*	3.1	16	8*	5.0
P. fluorescens	15	2	1.6	15	4	2.0	15	6*	3.0	15	10*	4.6
P. vulgaris	16	2	1.5	16	4	2.3	16	8	3.5	16	12	5.2
R. meliloti	16	2	1.8	16	6	2.5	15	8	4.0	15	15*	6.2
S. lipoferum	16	2	1.6	16	4	2.7	16	6	3.7	16	12*	5.8
Fusarium sp.	14	2	1.0	14	2	1.7	10	4*	2.5	10	8*	3.7
E atroseptica + *Fusarium*	15	2	1.0	14	2*	1.5	12	6*	2.3	12	10*	3.2
C. johnsonae + *Fusarium*	16	2	1.3	16	4*	1.9	16	6*	2.7	16	10*	3.6
Lysobacter + *Fusarium*	16	2	1.4	16	4*	2.0	16	6*	3.1	15	10*	4.2
P. fluorescens + *Fusarium*	15	2	1.2	15	4*	2.0	15	8*	3.0	15	10*	4.3
P. vulgaris + *Fusarium*	16	2	1.3	15	4*	1.8	15	6	3.0	15	12	4.8
R. meliloti + *Fusarium*	16	2	1.5	16	5*	2.2	16	8*	3.0	16	12*	5.1
S. lipoferum + *Fusarium*	16	2	1.5	16	6*	2.0	16	8*	3.3	16	10*	5.0
E. atroseptica + *Lysobacter*	16	2	1.4	15	4*	1.9	15	6*	2.7	13	10*	3.6
Lysobacter + *Cytophaga*	16	2	1.4	15	4*	2.1	15	6*	3.2	15	10*	4.1
E. atroseptica + *Lysobacter* + *Fusarium*	14	2	1.1	13	3*	1.6	10	4*	2.5	10	8*	3.3
Lysobacter + *Cytophaga* + *Fusarium*	15	2	1.3	15	4*	1.8	13	8*	2.9	13	12*	3.4

Table 10. Effect of combined bacterial pathogens + *Fusarium* on disease index and yield of alfalfa grown in the greenhouse.

Treatment	Disease index	Mean fresh seedling weight (g) (5x=±0.016)
UNWOUNDED		
Control	1	0.292
E. atroseptica	3	0.243
C. johnsonae	2	0.260
Lysobacter	2	0.241
P. fluorescens	2	0.252
P. vulgaris	1	0.256
R. meliloti	1	0.311
S. lipoferum	1	0.295
Fusarium	3	0.227
E. atroseptica + *Fusarium*	4	0.223
C. johnsonae + *Fusarium*	4	0.225
Lysobacter + *Fusarium*	4	0.250
P. fluorescens + *Fusarium*	4	0.250
P. vulgaris + *Fusarium*	3	0.259
R. meliloti + *Fusarium*	3	0.307
S. lipoferum + *Fusarium*	2	0.291
Lysobacter	4	0.229
Lysobacter + *Cytophaga*	3	0.243
E. atroseptica + *Lysobacter* + *Fusarium*	4	0.225
Lysobacter + *Cytophaga* + *Fusarium*	4	0.227
WOUNDED		
Control	1	0.289
E. atroseptica	3	0.262
C. johnsonae	2	0.249
Lysobacter	2	0.261
P. fluorescens	2	0.259
P. vulgaris	1	0.264
R. meliloti	1	0.302
S. lipoferum	1	0.290
Fusarium	3	0.231
E. atroseptica + *Fusarium*	4	0.220
C. johnsonae + *Fusarium*	4	0.227
Lysobacter + *Fusarium*	4	0.252
P. fluorescens + *Fusarium*	4	0.255
P. vulgaris + *Fusarium*	3	0.257
R. meliloti + *Fusarium*	3	0.257
S. lipoferum + *Fusarium*	3	0.259
E. atroseptica + *Lysobacter*	4	0.228
Lysobacter + *Cytophaga*	3	0.249
E. atroseptica + *Lysobacter* + *Fusarium*	4	0.223
Lysobacter + *Cytophaga* + *Fusarium*	4	0.225

important to keep in mind the possibility of antagonism and competition between organisms. Again, as in the previous experiments, wounded plants were poorer than unwounded. This supports the fact that phytopathogenic bacteria are opportunistic and require some type of prior injury to the host. In both the wounded and unwounded trials, plants inoculated with either *R. meliloti* or *S. lipoferum* grew better than did the controls. This is due to the nitrogen fixing capabilities of both these organisms. With respect to wounding, it appears that injury caused by fungi can serve as a mechanism by which bacteria gain entry into the plant. This can be seen by the similarities in survival rate of mechanically wounded plants as opposed to fungally wounded plants.

In most cases the degree of lesioning was greater when more than one pathogen was inoculated. Again enhancement of the disease capability of each organism was observed. Predictably the plant height and yield show a significant decrease when an efficient combination of pathogens is used. Typically *E. atroseptica* + *Fusarium* showed the greatest decrease in plant height and yield. This was followed by the combinations of *P. fluorescens*, *P. marginalis* or *C. johnsonae* with *Fusarium*, in decreasing order of severity. *Lysobacter*, when inoculated together with *Fusarium*, appears to have the least severe effect on plant height and yield. The affinity of *Lysobacter* for gram-negative bacteria and fungi has to be taken into account. Similar trends can be seen with the combination of *Cytophaga* with other pathogens. With both *Lysobacter* and *Cytophaga* the organisms are likely feeding simultaneously on the plant tissue and fellow pathogens, reducing the overall effect on the host plant.

The most damaging pathogen combination is that of a soft-rot *Erwinia* with *Fusarium*. Both produce large quantities of extracellular

enzymes and are efficient in macerating plant tissue. Given this combination one would expect that any plant subjected to these organisms would immediately succumb. This is not always the case. The degree to which the plant is affected depends on the overall health of the plant and the antagonistic effects that may develop between pathogens. A problem with using Hilson trays and an essentially unsterile growth cabinet, is that after a given period of time has elapsed, the population in the soil will not consist of one single type of organism. There will be cross-contamination by water splashing as plants are being watered, contamination from spore-forming organisms within the soil that were not killed by steam sterilization and contamination from the surrounding environment. However in nature a disease is seldom caused by just a single organism. Thus the interplay between naturally occurring rhizosphere and soil flora must be taken into account when looking at the overall disease picture. In practical terms the pathogenicity of individual organisms in highly controlled environments in the laboratory will not be observed to the same extent in nature.

F. Conclusions.

Common rhizosphere bacteria appear to be quite significant pathogens in root rot diseases. These organisms are opportunistic and require some form of prior injury to the host. This type of injury can take many forms and likely a number of factors are involved. Significant were wounds caused by other soil organisms - especially fungi and nematodes. The fungi can serve as pathogens in their own right but overall severity of disease appears to be enhanced by the activity of pectolytic, cellulolytic and saccharolytic bacteria. The concept that nematodes, feeding on roots, may be capable of injecting bacterial

pathogens into the root is novel and would bear further investigation to determine the role of nematodes in this disease complex.

The concept of synergistic pathogens in a disease complex is significant and may redefine the role of so-called saprophytic bacteria that are isolated from lesions. While these organisms may not produce cellulase or pectic enzymes, many are strongly saccharolytic and are capable of destroying starch reserves in the plant cell. This contributes to the overall debilitating effect on the plant. Genera such as *Chromobacterium* and *Serratia* appear to belong to this category of pathogen.

Lysobacter and *Cytophaga* species are an overlooked component in root rot diseases. Both are common rhizosphere organisms and contribute significantly to the cycling of organic matter in soil, especially cellulose. Because both these organisms possess such a wide range of macerative enzymes, they would fulfill the concept of an ideal plant pathogen. However both *Lysobacter* and *Cytophaga* may also serve in a regulatory capacity. Their affinity for other genera of bacteria, fungi and nematodes has been well-documented. Experiments conducted in this study indicate that both *Lysobacter* and *Cytophaga* can feed on other pathogens present as well as the plant host. This reduces the severity of the disease.

Other plants growing in association with alfalfa are important reservoirs for bacterial phytopathogens. Some isolates from this study came from lesions on the roots of common weeds growing in conjunction with alfalfa. As most alfalfa fields cannot be treated with herbicides, weeds do become a problem. The extent to which bacteria can infect plants growing in association with alfalfa bears further investigation.

Mechanical forces experienced by the growing plant can cause physical

injury to the root system, thus opening an avenue for entry of opportunistic pathogens. Especially important are the forces exerted by cycles of freezing and thawing within the soil. In northern temperate climates this type of injury can be very significant but will vary from year to year depending on the severity of the winter.

The concept of a disease complex is extremely significant, especially in root diseases. With the multiplicity of organisms, especially in the rhizosphere, combined with the physical and chemical nature of the soil in which the plant is growing, it seems trite to ascribe one single organism as the sole pathogen. A hololistic approach to plant disease is called for.

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APPENDIX 1

Source of Isolates

Isolate	Source
P-1	Isolated from lesioned alfalfa roots, Spruce Grove, Alberta
P-2	" " " " " " " "
P-3	" " " " " " " "
P-4	" " " " " " " "
P-5	" " " " " " " "
P-8	" " " " " St. Paul, Alberta
P-9	" " " " " " "
P-11	" " " " " " "
P-12	" " " " " " "
P-13	" " " " " Mallaig, Alberta
P-14	" " " " " " "
P-15	" " " " " " "
P-16	" " " " " " "
P-17	" " " " " " "
P-18	" " " " " " "
P-19	" " " " " Therien, Alberta
P-20	" " " " " " "
P-21	" " " " " " "
P-22	" " " " " St. Paul, Alberta
P-24	" " " " " " "
P-25	" " " " " " "
P-26	" " " " " " "
P-27	" " " " " " "
P-28	" " " " " " "
P-28	" " " rapeseed pod by Dr. A.W. Henry
P-29	" " " " " " "
P-30	" " " alfalfa roots, Mallaig, Alberta
P-31	" " " " " " "
P-32	" " " " " " "
P-33	" " " " " " "
P-34	" " " " " " "
P-35	" " " " " " "
E-1	" " " " " Spruce Grove, Alberta
E-2	" " " " " " " "
E-3	" " " " " " " "
E-4	" " " " " " " "
E-7	" " " " " " " "
E-8	" " " " " " " "
E-9	" " " " " " " "
E-10	" " " " " " " "
E-11	" " " " " " " "
E-12	" " " " " " " "
E-13	" " " " " " " "
E-17	Isolated from dandelion roots associated with alfalfa, Spruce Grove, Alberta
E-18	Isolated from lesioned alfalfa roots, Spruce Grove, Alberta
E-19	" " " " " " " "
E-20	" " " " " " " "
E-23	" " " " " St. Paul, Alberta
E-29	" " " " " " "
E-32	" " " " " " "
E-33	" " " " " " "
E-34	" " " " " " "
E-35	" " " " " " "
E-36	" " " " " " "
E-37	" " " " " " "
E-38	" " " " " " "
E-39	" " " " " " "
E-40	" " " " " " "
E-41	" " " " " " "
E-42	" " " " " " "
E-43	" " thistle roots associated with alfalfa
E-44	" " " " " " "

Source of Isolates (cont'd)

Isolate	Source
E-45	Isolated from lesioned alfalfa roots, St. Paul, Alberta
E-46	" " " " " " "
E-47	" " " " " Hallaig, Alberta
E-48	" " " " " " "
E-49	" " " " " " "
E-50	" " " " " " "
E-51	" " " " " " "
E-52	" " " " " " "
E-53	" " " " " " "
E-54	" " " " " " "
E-55	" " " " " " "
E-56	" " " " " " "
E-57	" " " " " " "
E-58	" " " " " " "
M12F-1	" " " alfalfa, fine roots, Morinville, Alberta
M12F-2	" " " " " " " "
M12F-3	" " " " " " " "
M12F-4	" " " " " " " "
M12F-5	" " " " " " " "
M12F-6	" " " " " " " "
M12F-7	" " " " " " " "
M12F-8	" " " " " " " "
M12F-9	" " " " " " " "
M12F-10	" " " " " " " "
M12F-11	" " " " " " " "
M12F-12	" " " " " " " "
M12F-13	" " " " " " " "
M12F-14	" " " " " " " "
M12C-1	" " " " crown, Morinville, Alberta
M12C-2	" " " " " " "
M12C-3	" " " " " " "
M12C-4	" " " " " " "
M12C-5	" " " " " " "
M12C-6	" " " " " " "
M12C-7	" " " " " " "
M12C-8	" " " " " " "
M12C-9	" " " " " " "
M12C-10	" " " " " " "
M12C-11	" " " " " " "
M12C-12	" " " " " " "
M12C-13	" " " " " " "
M12C-14	" " " " " " "
M12P-1	" " " " tap root, Morinville, Alberta
M12P-2	" " " " " " " "
M12P-3	" " " " " " " "
M12P-4	" " " " " " " "
M12P-5	" " " " " " " "
M12P-6	" " " " " " " "
M12P-7	" " " " " " " "
M12P-8	" " " " " " " "
M12P-9	" " dandelion associated with alfalfa, Morinville, Alberta
M11C-1	" " lesioned alfalfa, crown, Morinville, Alberta
M11C-2	" " " " " " "
M11C-3	" " " " " " "
M11C-4	" " " " " " "
M11C-5	" " " " " " "
M11C-6	" " " " " " "
M11C-7	" " " " " " "
Mt11P-1	" " " " tap root, Mayerthorpe, Alberta
Mt11P-2	" " " " " " " "
Mt11P-3	" " " " " " " "
Mt11P-4	" " " " " " " "
Mt11P-5	" " " " " " " "
Mt11P-6	" " " " " " " "
Mt11P-7	" " " " " " " "
Mt11P-8	" " " " " " " "
Mt11P-9	" " " " " " " "
Mt11P-10	" " " " " " " "

Source of Isolates (cont'd)

Isolate	Source
Mt11F-1	Isolated from lesioned alfalfa, fine roots, Mayerthorpe, Alberta
Mt11F-2	" " " " " " " "
Mt11F-3	" " " " " " " "
Mt11F-4	" " " " " " " "
Mt11F-5	" " " " " " " "
Mt11F-6	" " " " " " " "
Mt11F-7	" " " " " " " "
Mt11F-8	" " " " " " " "
Mt11F-9	" " " " " " " "
Mt11F-10	" " " " " " " "
Mt12F-1	" " " " " " " "
Mt12F-2	" " " " " " " "
Mt12F-3	" " " " " " " "
Mt12F-4	" " " " " " " "
Mt12F-5	" " " " " " " "
Mt12F-6	" " " " " " " "
Mt12F-7	" " " " " " " "
Mt12P-1	" " " " tap root, Mayerthorpe, Alberta
Mt12P-2	" " " " " " " "
Mt12P-2	" " " " " " " "
Mt12P-3	" " " " " " " "
Mt12P-4	" " " " " " " "
Mt12P-5	" " " " " " " "
Mt14P-1	Isolated from dandelion root associated with alfalfa
Mt14P-2	" " lesioned alfalfa, tap root, Mayerthorpe, Alberta
Mt14P-3	" " " " " " " "
Mt14P-4	" " " " " " " "
Mt14P-5	" " " " " " " "
Mt14P-6	" " " " " " " "
Mt14P-7	" " " " " " " "
Mt14P-8	" " " " " " " "
Mt14P-9	" " " " " " " "
Mt14P-10	" " " " " " " "
Mt14P-11	" " " " " " " "
Mt14P-12	" " " " " " " "
Mt14F-1	" " " alfalfa root, fine roots, Mayerthorpe
Mt14F-2	" " " " " " " "
Mt14F-3	" " " " " " " "
Mt14F-4	" " " " " " " "
Mt14F-5	" " " " " " " "
Mt14F-6	" " " " " " " "
Mt21C-1	" " " alfalfa, crown, Mayerthorpe
Mt21C-2	" " " " " "
Mt21C-3	" " " " " "
Mt21C-4	" " " " " "
Mt21C-5	" " " " " "
Mt21P-1	" " " " tap root, Mayerthorpe
Mt21P-2	" " " " " " "
Mt21P-3	" " " " " " "
Mt21P-4	" " " " " " "
Mt21P-5	" " " " " " "
Mt21P-6	" " " " " " "
Mt21P-7	" " " " " " "
Mt21F-1	" " " " fine roots, Mayerthorpe
Mt21F-2	" " " " " " "
Mt21F-3	" " " " " " "
Mt21F-4	" " " " " " "
Mt11P-1	" " " " tap root,
Mt11P-2	" " " " " " "
Mt11P-3	" " thistle root associated with alfalfa
Mt11P-4	" " lesioned alfalfa, tap root, Mayerthorpe
Mt11P-5	" " " " " " "
Mt11P-6	" " " " " " "
Mt11P-7	" " " " " " "
Mt11P-8	" " " " " " "
Mt11P-9	" " " " " " "
Mt11P-10	" " " " " " "

Source of Isolates (cont'd)

Isolate	Source
Mt11F-1	Isolated from lesioned alfalfa, fine roots, Mayerthorpe, Alberta
Mt11F-2	" " " " " " " "
Mt11F-3	" " " " " " " "
Mt11F-4	" " " " " " " "
Mt11F-5	" " " " " " " "
Mt11F-6	" " " " " " " "
Mt11F-7	" " " " " " " "
Mt11F-8	" " " " " " " "
Mt11F-9	" " " " " " " "
Mt11F-10	" " " " " " " "
Mt12F-1	" " " " " " " "
Mt12F-2	" " " " " " " "
Mt12F-3	" " " " " " " "
Mt12F-4	" " " " " " " "
Mt12F-5	" " " " " " " "
Mt12F-6	" " " " " " " "
Mt12F-7	" " " " " " " "
Mt12F-8	" " " " " " " "
Mt12P-1	" " " " tap root, " "
Mt12P-2	" " " " " " " "
Mt12P-3	" " " " " " " "
Mt12P-4	" " " " " " " "
Mt12P-5	" " " " " " " "
Mt14P-1	" " " " " " " "
Mt14P-2	" " " " " " " "
Mt14P-3	" " " " " " " "
Mt14P-3	" " " " " " " "
Mt14P-4	" " " " " " " "
Mt14P-5	" " " " " " " "
Mt14P-6	" " " " " " " "
Mt14P-7	" " " " " " " "
Mt14P-8	" " " " " " " "
Mt14P-9	" " " " " " " "
Mt14P-10	" " " " " " " "
Mt14P-11	" " " " " " " "
Mt14P-12	" " " " " " " "
Mt14F-1	" " " " fine roots, " "
Mt14F-2	" " " " " " " "
Mt14F-3	" " " " " " " "
Mt14F-4	" " " " " " " "
Mt14F-5	" " " " " " " "
Mt14F-6	" " " " " " " "
Mt21C-1	" " " " crown, " "
Mt21C-1	" " " " " " " "
Mt21C-2	" " " " " " " "
Mt21C-3	" " " " " " " "
Mt21C-4	" " " " " " " "
Mt21C-5	" " " " " " " "
Mt21P-1	" " " " primary roots, " "
Mt21P-2	" " " " " " " "
Mt21P-3	" " " " " " " "
Mt21P-4	" " " " " " " "
Mt21P-5	" " " " " " " "
Mt21P-6	" " " " " " " "
Mt21P-7	" " " " " " " "
Mt21F-1	" " " " fine roots " "
Mt21F-2	" " " " " " " "
Mt21F-3	" " " " " " " "
Mt21F-4	" " " " " " " "
Mt21F-5	" " " " " " " "
Mt21F-6	" " " " " " " "
Mt21F-7	" " " " " " " "
Mt82F-1	" " " " " " " "
Mt82F-2	" " " " " " " "
Mt82F-3	" " " " " " " "
Mt82F-4	" " " " " " " "
Mt82F-5	" " " " " " " "
Mt82F-6	" " " " " " " "
Mt82F-7	" " " " " " " "
Mt82F-8	" " " " " " " "
Mt82F-9	" " " " " " " "
Mt82F-10	" " " " " " " "

Source of Isolates (cont'd)

Isolate	Source
Mt23F-1	Isolated from lesioned alfalfa, fine roots, Mayerthorpe, Alberta
Mt23F-2	" " " " " " " "
Mt23F-3	" " " " " " " "
Mt23F-4	" " " " " " " "
Mt23F-5	" " " " " " " "
Mt23F-6	" " " " " " " "
Mt23F-7	" " " " " " " "
Mt23F-8	" " " " " " " "
Mt23F-9	" " " " " " " "
Mt23F-10	" " " " " " " "
Mt23F-11	" " " " " " " "
Mt23P-1	" " " " tap roots, " "
Mt23P-2	" " " " " " " "
Mt23P-3	" " " " " " " "
Mt23P-4	" " " " " " " "
Mt23C-1	" " " " crown, " "
Mt23C-2	" " " " " " " "
Mt23C-3	" " " " " " " "
Mt23C-4	" " " " " " " "
Mt23C-5	" " " " " " " "
Mt84F-1	" " " " fine roots, " "
Mt84F-2	" " " " " " " "
Mt84F-3	" " " " " " " "
Mt84F-4	" " " " " " " "
Mt84F-5	" " " " " " " "
Mt84F-6	" " " " " " " "
Mt84P-1	" " " " tap root, " "
Mt84P-2	" " " " " " " "
Mt84P-3	" " " " " " " "
Sp1-1	" " " alfalfa roots, St. Paul, Alberta
Sp1-2	" " " " " " "
Sp1-13	" " " " " " "
Sp1-4	" " " " " " "
Sp1-5	" " " " " " "
Sp1-6	" " " " " " "
Sp1-9	" " " " " " "
Sp2	" " " " " " "

Appendix 2-1. Physiology and biochemistry of *Bacillus polymyxa* isolated from alfalfa root lesions.

	Morphology				Physiology								Sugars								
					NaCl tolerance		O_2 requirement			Temperature °C											
	Shape	Gram	Motility	Flagella	5%	7%	Aerobic	Anaerobic	Spores	Min.	Max.	Opt.	Arabinose	Cellobiose	Fructose	Glucose	Lactose	Mannose	Maltose	Raffinose	Sucrose	Xylose
B. polymyxa																					
E-11	rod	+	+	Peri.*	-	-	+	+	+	5	38	24	+		+	+	+	+	+	+	+	+
M12F-4	rod	+	+	"	-	-	+	+	+	5	38	24	+		+	+	+	+	+	+	+	+
M12F-7	rod	+	+	"	-	-	+	+	+	5	40	25	+		+	+	+	+	+	+	+	+
M12F-11	rod	+	+	"	-	-	+	+	+	5	40	25	+		+	+	+	+	+	+	+	+
M12P-4	rod	+	+	"	-	-	+	+	+	3	38	23	+		+	+	+	+	+	+	+	+
MT12P-3	rod	+	+	"	-	-	+	-	+	5	40	25	+		+	+	+	+	+	+	+	+
MT12P-5	rod	+	+	"	-	-	+	-	+	5	40	25	+		+	+	+	+	+	+	+	+
MT14P-9	rod	+	+	"	-	-	+	-	+	4	38	25	+		+	+	+	+	+	+	+	+
MT21F-4	rod	+	+	"	-	-	+	-	+	4	38	25	+		+	+	+	+	+	+	+	+
MT11P-5	rod	+	+	"	-	-	+	-	+	5	40	23	+		+	+	+	+	+	+	+	+
MT11P-6	rod	+	+	"	-	-	+	-	+	5	40	23	+		+	+	+	+	+	+	+	+
MT12P-2	rod	+	+	"	-	-	+	-	+	5	40	23	+		+	+	+	+	+	+	+	+
MT12P-5	rod	+	+	"	-	-	+	-	+	4	38	21	+		+	+	+	+	+	+	+	+
MT21F-4	rod	+	+	"	-	-	+	-	+	5	40	23	+		+	+	+	+	+	+	+	+
MT23F-4	rod	+	+	"	-	-	+	-	+	5	40	25	+		+	+	+	+	+	+	+	+
SP1-1	rod	+	+	"	-	-	+	-	+	3	35	23	+		+	+	+	+	+	+	+	+
SP1-4	rod	+	+	"	-	-	+	-	+	3	38	23	+		+	+	+	+	+	+	+	+

Appendix 2-1 (cont'd).

	Alcohols		Polysaccharides		Miscellaneous biochemistry											Action on pectate gel		
	Ethanol	Mannitol	Cellulose	Pectin	Arginine Dihydrolase	Catalase	Citrate	Gelatin Liquefaction	Indole	M.R.	NO_3 + NO_2	Oxidase	Pigments	Starch hydrolysis	Urease	V.P.	pH 5	pH 6.8	pH 8
B. polymyxa																		
E-11	+	+	-	+	-	+	-	+	-	+	-	None	+	-	+	+	+	-
M12F-4	+	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
M12F-7	+	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
M12F-11	+	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
M12P-4	+	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
mt12P-3	±	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
MT12P-5	±	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
MT14P-9	+	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
MT21F-4	+	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
MT11P-5	+	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
MT11P-6	±	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
MT12P-2	±	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
MT12P-5	±	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
MT21F-4	±	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
MT23F-6	+	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
SP1-1	+	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-
SP-4	+	+	-	+	-	+	-	+	-	+	-	"	+	-	+	+	+	-

Appendix 2-2. Physiology and biochemistry of *Clostridium* and *Chromobacterium violaceum* isolated from alfalfa root lesions.

	Morphology				Physiology								Sugars									
					NaCl tolerance		O_2 requirement			Temperature °C												
	Shape	Gram	Motility	Flagella	5%	7%	Aerobic	Anaerobic	Spores	Min.	Max.	Opt.	Arabinose	Cellobiose	Fructose	Glucose	Lactose	Mannose	Maltose	Raffinose	Sucrose	Xylose
Clostridium																						
M12C-10	rod	+	+	Peri.	-	-	-	+	+	5	38	25	-	+	+	+	+	+	+	+	+	+
MT12P-2	rod	+	+	"	-	-	-	+	+	5	38	25	-	+	+	+	+	+	+	+	+	+
MT14P-3	rod	+	+	"	-	-	-	+	+	5	38	25	-	+	+	+	+	+	+	+	+	+
MT11P-9	rod	+	+	"	-	-	-	+	+	5	40	25	-	+	+	+	+	+	+	+	+	+
SP3-1	rod	+	+	"	-	-	-	+	+	5	40	25	-	+	+	+	+	+	+	+	+	+
SP4	rod	+	+	"	-	-	-	+	+	5	40	25	-	+	+	+	+	+	+	+	+	+
Chromobacterium violaceum																						
E-33	rod	-	+	Polar	+	-	+	+	-	4	38	23	-	-	+	+	-	+	+	-	+	±
E-53	rod	-	+	"	+	-	+	+	-	4	38	23	-	-	+	+	-	+	+	-	+	±
M12F-12	rod	-	+	"	+	-	+	+	-	4	38	23	-	-	+	+	-	+	-	-	+	-
MT14P-8	rod	-	+	"	+	-	+	+	-	4	38	23	-	-	+	+	-	+	±	-	+	-
MT14F-4	rod	-	+	"	+	-	+	+	-	4	38	23	-	-	+	+	-	+	±	-	+	-
MT14F-6	rod	-	+	"	+	-	+	+	-	2	40	23	-	-	+	+	-	+	±	-	+	-
MT11P-8	rod	-	+	"	+	-	+	+	-	4	38	23	-	-	+	+	-	+	±	-	+	-
MT14F-6	rod	-	+	"	+	-	+	+	-	5	38	23	+	-	+	+	-	+	+	-	+	-
MT82F-5	rod	-	+	"	+	-	+	+	-	5	38	23	+	-	+	+	-	+	+	-	+	-

Appendix 2-2 (Cont'd)

	Alcohols		Polysaccharides		Miscellaneous biochemistry												Action on pectate gel		
	Ethanol	Mannitol	Cellulose	Pectin	Arginine Dihydrolase	Catalase	Citrate	Gelatin Liquefaction	Indole	M.R.	$NO_3 \rightarrow NO_2$	Oxidase	Pigments	Starch hydrolysis	Urease	V.P.	pH 5	pH 6.8	pH 8
Clostridium																			
M12C-10	-	-	+	+	-	-	-	-	-	-	-	-	None	+	-	+	-	+	-
MT12P-2	-	-	+	+	-	-	-	-	-	-	-	-	"	+	-	+	-	+	-
MT14P-3	-	-	+	+	-	-	-	-	-	-	-	-	"	+	-	+	-	+	-
MT11P-9	-	-	+	+	-	-	-	-	-	-	-	-	"	+	-	+	-	+	-
SP3-1	-	-	+	+	-	-	-	-	-	-	-	-	"	+	-	+	-	+	-
SP4	-	-	+	+	-	-	-	-	-	-	-	-	"	+	-	+	-	+	-
Chromobacterium violaceum																			
E-33	-	±	-	-	-	+	+	+	-	-	+	-	Violacein	+	-	-	-	-	-
E-53	-	±	-	-	-	+	+	+	-	-	+	-	"	+	-	-	-	-	-
M12F-12	-	-	-	-	-	+	+	+	-	-	+	-	"	+	-	-	-	-	-
MT14P-8	-	-	-	-	-	+	+	+	-	-	+	-	"	+	-	-	-	-	-
MT14F-4	-	-	-	-	-	+	+	+	-	-	+	-	"	+	-	-	-	-	-
MT14F-6	-	-	-	-	-	+	+	+	-	-	+	-	"	+	-	-	-	-	-
MT11P-8	-	-	-	-	-	+	+	+	-	-	+	-	"	+	-	-	-	-	-
MT14F-6	-	+	-	-	-	+	+	+	-	-	+	-	"	+	-	-	-	-	-
MT82F-5	-	+	-	-	-	+	+	+	-	-	+	-	"	+	-	-	-	-	-

Appendix 2-3. Physiology and biochemistry of *Cytophaga johnsonae* isolated from alfalfa root lesions.

	Morphology				Physiology								Sugars*									
					NaCl tolerance		O_2 requirement			Temperature °C												
	Shape	Gram	Motility	Flagella	5%	7%	Aerobic	Anaerobic	Spores	Min.	Max.	Opt.	Arabinose	Cellobiose	Fructose	Glucose	Lactose	Mannose	Maltose	Raffinose	Sucrose	Xylose
Cytophaga johnsonae																						
P-17	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
E-3	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
E-40	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
E-41	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
M11F-1	rod	-	+	-	-	-	+	-	-	5	38	23	+	+		+	-	-	+	-	-	+
M12F-1	rod	-	+	-	-	-	+	-	-	5	38	23	+	+		+	-	-	+	-	-	+
M12F-2	rod	-	+	-	-	-	+	-	-	5	38	23	+	+		+	-	-	+	-	-	+
M12F-8	rod	-	+	-	-	-	+	-	-	3	40	23	+	+		+	-	-	+	-	-	+
M12C-2	rod	-	+	-	-	-	+	-	-	3	40	23	+	+		+	-	-	+	-	-	+
M12C-8	rod	-	+	-	-	-	+	-	-	3	30	23	+	+		+	-	-	+	-	-	+
M12C-11	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
M12C-14	rod	-	+	-	-	-	+	-	-	5	38	23	+	+		+	-	-	+	-	-	+
M12P-3	rod	-	+	-	-	-	+	-	-	5	38	23	+	+		+	-	-	+	-	-	+
M12P-9	rod	-	+	-	-	-	+	-	-	3	40	23	+	+		+	-	-	+	-	-	+
MT11P-2	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT11P-3	rod	-	+	-	-	-	+	-	-	5	38	23	+	+		+	-	-	+	-	-	+
mt11P-5	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT11P-6	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT11P-7	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT11P-8	rod	-	+	-	-	-	+	-	-	5	40	23	+	+		+	-	-	+	-	-	+
MT12F-1	rod	-	+	-	-	-	+	-	-	5	40	23	+	+		+	-	-	+	-	-	+
MT12F-2	rod	-	+	-	-	-	+	-	-	5	40	23	+	+		+	-	-	+	-	-	+
MT12F-3	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT12F-4	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+

Appendix 2-3 (cont'd)

	Alcohols		Polysaccharides		Miscellaneous biochemistry										Action on pectate gel				
	Ethanol	Mannitol	Cellulose	Pectin	Arginine Dihydrolase	Catalase	Citrate	Gelatin Liquefaction	Indole	M.R.	NO_3 + NO_2	Oxidase	Pigments	Starch hydrolysis	Urease	V.P.	pH 5	pH 6.8	pH 8
Cytophaga johnsonae																			
P-17	-	-	+	+	-	+	-	+	-	-	+	-	Yellow	+	-	-	-	+	+
E-3	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
E-40	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
E-41	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
M11F-1	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
M12F-1	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
M12F-2	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
M12F-8	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
M12C-2	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
M12C-8	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
M12C-11	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
M12C-14	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
M12P-3	-	-	+	+	-	+	-	+	-	-	+	-	Orange	+	-	-	-	+	+
M12P-6	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
M12P-9	-	-	+	+	-	+	-	+	-	-	+	-	Yellow	+	-	-	-	+	+
MT11P-2	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT11P-3	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT11P-5	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT11P-6	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT11P-7	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT11P-8	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT12F-1	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT12F-2	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT12F-3	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT12F-4	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+

Appendix 2-3. (cont'd)

	Morphology						Physiology							Sugars								
					NaCl tolerance		O_2 requirement			Temperature °C												
	Shape	Gram	Motility	Flagella	5%	7%	Aerobic	Anaerobic	Spores	Min.	Max.	Opt.	Arabinose	Cellobiose	Fructose	Glucose	Lactose	Mannose	Maltose	Raffinose	Sucrose	Xylose
Cytophaga johnsonae																						
MT12F-5	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT12F-6	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT12F-7	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT12P-4	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT14F-1	rod	-	+	-	-	-	+	-	-	5	38	23	+	+		+	-	-	+	-	-	+
MT21C-1	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT21C-2	rod	-	+	-	-	-	+	-	-	3	39	25	+	+		+	-	-	+	-	-	+
MT21P-5	rod	-	+	-	-	-	+	-	-	5	40	25	+	+		+	-	-	+	-	-	+
MT21P-6	rod	-	+	-	-	-	+	-	-	5	38	23	+	+		+	-	-	+	-	-	+
MT21F-1	rod	-	+	-	-	-	+	-	-	3	38	25	+	+		+	-	-	+	-	-	+
MT21F-2	rod	-	+	-	-	-	+	-	-	3	38	25	+	+		+	-	-	+	-	-	+
MT21F-3	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT11F-8	rod	-	+	-	-	-	+	-	-	5	40	25	+	+		+	-	-	+	-	-	+
MT12F-7	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT12F-8	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT12P-4	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT82F-1	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT82F-2	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT82F-3	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT82F-8	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT23F-2	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT23P-1	rod	-	+	-	-	-	+	-	-	5	38	23	+	+		+	-	-	+	-	-	+
MT23P-2	rod	-	+	-	-	-	+	-	-	5	38	23	+	+		+	-	-	+	-	-	+
MT23P-4	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT23C-1	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT23C-2	rod	-	+	-	-	-	+	-	-	5	40	23	+	+		+	-	-	+	-	-	+
MT23C-3	rod	-	+	-	-	-	+	-	-	5	40	23	+	+		+	-	-	+	-	-	+
MT23C-4	rod	-	+	-	-	-	+	-	-	3	38	23	+	+		+	-	-	+	-	-	+
MT84F-5	rod	-	+	-	-	-	+	-	-	4	38	25	+	+		+	-	-	+	-	-	+
MT84F-6	rod	-	+	-	-	-	+	-	-	4	38	25	+	+		+	-	-	+	-	-	+
MT84P-1	rod	-	+	-	-	-	+	-	-	4	38	23	+	+		+	-	-	+	-	-	+
SP5-2	rod	-	+	-	-	-	+	-	-	4	38	25	+	+		+	-	-	+	-	-	+

Appendix 2-4. (cont'd)

	Alcohols		Polysaccharides		Miscellaneous biochemistry												Action on pectate gel		
	Ethanol	Mannitol	Cellulose	Pectin	Arginine Dihydrolase	Catalase	Citrate	Gelatin Liquefaction	Indole	M.R.	$NO_3 \rightarrow NO_2$	Oxidase	Pigments	Starch hydrolysis	Urease	V.P.	pH 5	pH 6.8	pH 8
E. atroseptica																			
P-14	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
P-20	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
P-27	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
P-30	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
E-2	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
E-4	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
E-7	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
E-12	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
E-17	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
E-45	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
E-54	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
E-58	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
M12F-10	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
M12C-5	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
M12C-6	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
M12C-7	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
M12C-12	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
M12C-13	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
MT21P-1	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
MT11F-9	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
SP-2	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-
SP-3	-	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	+	-

Appendix 2-3. (cont'd)

	Alcohols		Polysaccharides		Miscellaneous biochemistry												Action on pectate gel		
	Ethanol	Mannitol	Cellulose	Pectin	Arginine Dihydrolase	Catalase	Citrate	Gelatin Liquefaction	Indole	M.R.	$NO_3 \rightarrow NO_2$	Oxidase	Pigments	Starch hydrolysis	Urease	V.P.	pH 5	pH 6.8	pH 8
Cytophaga johnsonae																			
MT12F-5	-	-	+	+	-	+	-	+	-	-	+	-	Yellow	+	-	-	-	+	±
MT12F-6	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT12F-7	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT12P-4	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT14F-1	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	±
MT21C-1	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	±
MT21C-2	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT21P-5	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT21P-6	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT21F-1	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT21F-2	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT21F-3	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	±
MT11F-8	-	-	+	+	-	+	-	+	-	-	+	-	Orange	+	-	-	-	+	±
MT12F-7	-	-	+	+	-	+	-	+	-	-	+	-	Yellow	+	-	-	-	+	±
MT12F-8	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT12P-4	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT82F-1	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT82F-2	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT82F-3	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT82F-8	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT23F-2	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT23P-1	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	±
MT23P-2	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	±
MT23P-4	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT23C-1	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT23C-2	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+
MT23C-3	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	±
MT23C-4	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	±
MT84F-5	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	±
MT84F-6	-	-	+	+	-	+	-	+	-	-	+	-	Orange	+	-	-	-	+	+
MT84P-1	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	±
SP5-2	-	-	+	+	-	+	-	+	-	-	+	-	"	+	-	-	-	+	+

Appendix 2-5. Physiology and biochemistry of *Erwinia carotovora* isolated from alfalfa root lesions.

	Morphology				Physiology								Sugars									
					NaCl tolerance		O_2 requirement			Temperature °C												
	Shape	Gram	Motility	Flagella	5%	7%	Aerobic	Anaerobic	Spores	Min.	Max.	Opt.	Arabinose	Cellobiose	Fructose	Glucose	Lactose	Mannose	Maltose	Raffinose	Sucrose	Xylose
E. carotovora																						
E-1	rod	-	+	Peri.	+	+	+	+	-	6	40	25	+	+	+	+	+	±	±	+	+	+
E-10	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	±	±	+	+	+
E-13	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	±	-	+	+	+
E-18	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	-	-	+	+	+
E-29	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	±	±	+	+	+
E-32	rod	-	+	"	+	+	+	+	-	6	38	25	+	+	+	+	+	±	±	+	+	+
E-34	rod	-	+	"	+	+	+	+	-	6	38	25	+	+	+	+	+	±	±	+	+	+
E-35	rod	-	+	"	+	+	+	+	-	6	38	25	+	+	+	+	+	±	±	+	+	+
E-36	rod	-	+	"	+	+	+	+	-	6	38	25	+	+	+	+	+	-	-	+	+	+
E-43	rod	-	+	"	+	+	+	+	-	6	38	25	+	+	+	+	+	-	-	+	+	+
E-44	rod	-	+	"	+	+	+	+	-	6	38	25	+	+	+	+	+	±	±	+	+	+
E-46	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	-	-	+	+	+
E-48	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	-	-	+	+	+
E-50	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	-	-	+	+	+
E-51	rod	-	+	"	+	+	+	+	-	6	38	25	+	+	+	+	+	-	±	+	+	+
E-56	rod	-	+	"	+	+	+	+	-	6	38	25	+	+	+	+	+	±	±	+	+	+
M12F-5	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	±	±	+	+	+
M12F-6	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	-	-	+	+	+
M12F-9	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	-	-	+	+	+
M12F-13	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	±	±	+	+	+
M12P-2	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	±	±	+	+	+
M12P-5	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	±	±	+	+	+
M12P-7	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	-	-	+	+	+
M12P-8	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	-	-	+	+	+
MT21C-3	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	-	-	+	+	+
SP1-1	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	-	-	+	+	+
SP6-2	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	±	-	+	+	+
SP6-6	rod	-	+	"	+	+	+	+	-	6	40	25	+	+	+	+	+	±	±			

Appendix 2-5. (cont'd)

	Alcohols		Polysaccharides		Miscellaneous biochemistry										Action on pectate gel					
	Ethanol	Mannitol	Cellulose	Pectin	Arginine Dihydrolase	Catalase	Citrate	Gelatin Liquefaction	Indole	M.R.	$NO_3 \rightarrow NO_2$	Oxidase	Pigments	Starch hydrolysis	Urease	V.P.	pH 5	pH 6.8	pH 8
E. carotovora																			
E-1	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-10	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-13	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-18	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-29	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-32	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-34	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-35	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-36	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-43	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-44	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-46	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-48	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-50	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-51	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
E-56	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
M12F-5	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
M12F-6	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
M12F-9	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
M12F-13	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
M12P-2	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
M12P-5	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
M12P-7	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
M12P-8	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
MT21C-3	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
SP1-1	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
SP6-2	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
SP6-6	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-

Appendix 2-6. Physiology and biochemistry of *Lysobacter enzymogenes* isolated from alfalfa root lesions.

	Morphology				Physiology								Sugars									
					NaCl tolerance		O_2 requirement			Temperature °C												
	Shape	Gram	Motility	Flagella	5%	7%	Aerobic	Anaerobic	Spores	Min.	Max.	Opt.	Arabinose	Cellobiose	Fructose	Glucose	Lactose	Mannose	Maltose	Raffinose	Sucrose	Xylose
Lysobacter enzymogenes																						
E37	rod	-	+	-	-	-	+	-	-	4	40	25	-	-	-	+	-	-	-	-	+	-
E39	rod	-	+	-	-	-	+	-	-	4	40	23	-	-	-	+	-	-	-	-	+	-
M12C-1	rod	-	+	-	-	-	+	-	-	4	40	23	-	-	-	+	-	-	-	-	+	-
MT82F-9	rod	-	+	-	-	-	+	-	-	4	40	23	-	-	-	+	-	±	-	-	+	±
MT82F-10	rod	-	+	-	-	-	+	-	-	4	40	23	-	-	-	+	-	±	-	-	+	±
MT23F-1	rod	-	+	-	-	-	+	-	-	4	40	23	-	-	-	+	-	±	-	-	+	±
MT23F-5	rod	-	+	-	-	-	+	-	-	4	40	23	-	-	-	+	-	-	-	-	+	-
MT23F-8	rod	-	+	-	-	-	+	-	-	4	40	21	-	-	-	+	-	-	-	-	+	-
MT23F-9	rod	-	+	-	-	-	+	-	-	4	40	21	-	-	-	+	-	-	-	-	+	-
MT23F-11	rod	-	+	-	-	-	+	-	-	4	40	21	-	-	-	+	-	-	-	-	+	-
SP1-9	rod	-	+	-	-	-	+	-	-	4	40	23	-	-	-	+	-	±	-	-	+	±
SP6-3	rod	-	+	-	-	-	+	-	-	4	40	23	-	-	-	+	-	-	-	-	+	-
SP7-1	rod	-	+	-	-	-	+	-	-	4	40	23	-	-	-	+	-	-	-	-	+	-

Appendix 2-6 (cont'd)

	Alcohols		Polysaccharides		Miscellaneous biochemistry												Action on pectate gel		
	Ethanol	Mannitol	Cellulose	Pectin	Arginine Dihydrolase	Catalase	Citrate	Gelatin Liquefaction	Indole	M.R.	NO_3 + NO_2	Oxidase	Pigments	Starch hydrolysis	Urease	V.P.	pH 5	pH 6.8	pH 8
Lysobacter enzymogenes																			
E-37	-	-	+	+	-	+	-	+	-	-	+	+	brown	+	-	-	-	+	±
E-39	-	-	+	+	-	+	-	+	-	-	+	+	green-brown	+	-	-	-	+	±
M12C-1	-	-	+	+	-	+	-	+	-	-	+	+	brown	+	-	-	-	+	±
MT82F-9	-	-	+	+	-	+	-	+	-	-	+	+	"	+	-	-	-	+	-
MT82F-10	-	-	+	+	-	+	-	+	-	-	+	+	"	+	-	-	-	+	-
MT23F-1	-	-	+	+	-	+	-	+	-	-	+	+	"	+	-	-	-	+	±
MT23F-5	-	-	+	+	-	+	-	+	-	-	+	+	"	+	-	-	-	+	-
MT23F-8	-	-	+	+	-	+	-	+	-	-	+	+	"	+	-	-	-	+	-
MT23F-9	-	-	+	+	-	+	-	+	-	-	+	+	"	+	-	-	-	+	-
MT23F-11	-	-	+	+	-	+	-	+	-	-	+	+	"	+	-	-	-	+	-
SP1-9	-	-	+	+	-	+	-	+	-	-	+	+	"	+	-	-	-	+	±
SP6-3	-	-	+	+	-	+	-	+	-	-	+	+	"	+	-	-	-	+	-
SP6-1	-	-	+	+	-	+	-	+	-	-	+	+	"	+	-	-	-	+	-

Appendix 2-7. Physiology and biochemistry of *Pseudomonas fluorescens* isolated from alfalfa root lesions.

	Morphology				Physiology								Sugars									
					NaCl tolerance		O_2 requirement			Temperature °C												
	Shape	Gram	Motility	Flagella	5%	7%	Aerobic	Anaerobic	Spores	Min.	Max.	Opt.	Arabinose	Cellobiose	Fructose	Glucose	Lactose	Mannose	Maltose	Raffinose	Sucrose	Xylose
P. fluorescens																						
P-4	rod	-	+	polar	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	+	+	+
P-8	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	+	+	+
P-9	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	+	+	+
P-11	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	+	+	+
P-13	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	±	+	+
P-15	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	±	-	+
P-18	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	±	-	+
P-19	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	+	-	+
P-22	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	+	-	+
P-26	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	+	-	+
P-29	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	±	-	+
P-31	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	±	-	+
P-32	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	+	-	+
P-33	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	±	-	+
P-34	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	-	-	+
E-16	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	±	-	+
E-23	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	±	-	+
E-38	rod	-	+	"	+	-	+	-	-	2	38	23	+	+	+	+	-	+	+	±	-	+
E-42	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	-	-	+
E-52	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	-	-	+
M12C-3	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	±	-	+
M12C-4	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	±	-	+
M12C-9	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	±	-	+
M12P-1	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	±	-	+
M11C-3	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	±	-	+
M11C-4	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	-	-	+
M11C-5	rod	-	+	"	+	-	+	-	-	2	38	23	+	+	+	+	-	+	+	-	-	+
MT11P-1	rod	-	+	"	+	-	+	-	-	2	40	23	+	+	+	+	-	+	+	-	-	+
MT11P-4	rod	-	+	"	+	-	+	-	-	2	40	23	+	±	+	+	-	+	+	-	-	+
MT11F-1	rod	-	+	"	+	-	+	-	-	2	38	23	+	+	+	+	-	+	+	-	-	+
MT11F-2	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	-	-	+
MT11F-3	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	±	-	+
MT11F-4	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	±	-	+
MT11F-5	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	±	-	+

Appendix 2-7. (Cont'd)

	Alcohols		Polysaccharides		Miscellaneous biochemistry										Action on pectate gel		
	Ethanol	Mannitol	Cellulose	Pectin	Arginine Dihydrolase	Catalase	Citrate	Gelatin Liquefaction	Indole	M.R.	$NO_3 \rightarrow NO_2$	Oxidase	Pigments	Starch hydrolysis	Urease	V.P.	pH 5	pH 6.8	pH 8
P. fluorescens																			
P-4	-	+	-	+	+	+	+	+	-		+	+	fluorescent	-	+		-	+	-
P-8	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-9	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-11	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-13	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-15	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-18	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-19	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-22	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-26	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-29	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-31	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-32	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-33	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
P-34	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
E-16	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
E-23	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
E-38	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
E-42	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
E-52	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
M12C-3	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
M12C-4	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
M12C-9	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
M12P-1	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
M11C-3	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
M11C-4	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
M11C-5	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT11P-1	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT11P-4	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT11F-1	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT11F-2	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-

Appendix 2-7. (Cont'd)

	Morphology				Physiology								Sugars									
					NaCl tolerance		O_2 requirement			Temperature °C												
	Shape	Gram	Motility	Flagella	5%	7%	Aerobic	Anaerobic	Spores	Min.	Max.	Opt.	Arabinose	Cellobiose	Fructose	Glucose	Lactose	Mannose	Maltose	Raffinose	Sucrose	Xylose
P. fluorescens																						
MT11F-6	rod	-	+	polar	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	±	-	+
MT11F-7	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	-	-	+
MT11F-10	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	+	-	+
MT14P-1	rod	-	+	"	+	-	+	-	-	2	40	25	+	-	+	+	-	+	+	±	-	+
MT14P-2	rod	-	+	"	+	-	+	-	-	2	40	25	+	-	+	+	-	+	+	±	-	+
MT14P-5	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	-	-	+
MT14P-6	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	-	-	+
MT14P-7	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	±	-	+
MT14P-10	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	-	-	+
MT14P-11	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	±	-	+
MT14P-12	rod	-	+	"	+	-	+	-	-	2	38	23	+	+	+	+	-	+	+	+	-	+
MT14F-1	rod	-	+	"	+	-	+	-	-	2	38	23	+	+	+	+	-	+	+	-	-	+
MT14F-2	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	-	-	+
MT14F-3	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	-	-	+
MT21C-4	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	-	-	+
MT21C-5	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	-	-	+
MT21P-2	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	±	-	+
MT21P-3	rod	-	+	"	+	-	+	-	-	2	38	23	+	+	+	+	-	+	+	+	-	+
MT21P-4	rod	-	+	"	+	-	+	-	-	2	40	23	+	±	+	+	-	+	+	+	-	+
MT21F-5	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	±	-	+
MT82F-4	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	-	-	+
MT82F-6	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	-	-	+
MT82F-7	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	-	+	+	-	-	+
MT23F-3	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	±	-	+
MT23F-4	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	-	+	+	±	-	+
MT23F-10	rod	-	+	"	+	-	+	-	-	2	38	23	+	+	+	+	-	+	+	-	-	+
MT84F-1	rod	-	+	"	+	-	+	-	-	2	38	23	+	+	+	+	-	+	+	-	-	+
MT84F-2	rod	-	+	"	+	-	+	-	-	2	38	23	+	+	+	+	-	+	+	±	-	+
MT84F-3	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	+	-	+	±	-	+
MT84F-4	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	+	-	+	±	-	+
MT84P-2	rod	-	+	"	+	-	+	-	-	2	40	23	+	-	+	+	+	-	+	+	-	+
MT84P-3	rod	-	+	"	+	-	+	-	-	2	40	23	+	-	+	+	+	-	+	+	-	+
SP1-13	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	+	-	+	±	-	+
SP1-5	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	+	-	+	-	-	+
SP1-6	rod	-	+	"	+	-	+	-	-	2	38	23	+	±	+	+	+	-	+	-	-	+
SP1-8	rod	-	+	"	+	-	+	-	-	2	40	23	+	-	+	+	+	-	+	-	-	+
SP6-4	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	+	-	+	±	-	+
SP6-5	rod	-	+	"	+	-	+	-	-	2	38	23	+	-	+	+	+	-	+	-	-	+

Appendix 2-7 (Cont'd)

	Alcohols		Polysaccharides		Miscellaneous biochemistry										Action on pectate gel		
	Ethanol	Mannitol	Cellulose	Pectin	Arginine Dihydrolase	Catalase	Citrate	Gelatin Liquefaction	Indole	M.R.	$NO_3 \rightarrow NO_2$	Oxidase	Pigments	Starch hydrolysis	Urease	V.P.	pH 5	pH 6.8	pH 8
P fluorescens																			
MT11F-6	-	+	-	+	+	+	+	+	-		+	+	fluorescent	-	+		-	+	-
MT11F-7	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT11F-10	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT14P-1	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT14P-2	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT14P-5	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT14P-6	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT14P-7	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT14P-10	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT14P-11	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT14P-12	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT14F-1	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT14F-2	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT14F-3	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT21C-4	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT21C-5	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT21P-2	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT21P-3	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT21P-4	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT21F-5	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT82F-4	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT82F-6	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT82F-7	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT23F-3	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT23F-4	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT23F-10	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT84F-1	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT84F-2	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT84F-3	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT84F-4	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT84P-2	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
MT84P-3	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
SP1-33	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
SP1-5	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
SP1-6	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
SP1-8	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
SP6-4	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-
SP6-5	-	+	-	+	+	+	+	+	-		+	+	"	-	+		-	+	-

Appendix 2-8. Physiology and biochemistry of *Pseudomonas marginalis* isolated from alfalfa root lesions.

	Morphology				Physiology								Sugars									
					NaCl tolerance		O_2 requirement			Temperature °C												
	Shape	Gram	Motility	Flagella	5%	7%	Aerobic	Anaerobic	Spores	Min.	Max.	Opt.	Arabinose	Cellobiose	Fructose	Glucose	Lactose	Mannose	Maltose	Raffinose	Sucrose	Xylose
P. marginalis																						
P-12	rod	-	+	Polar	+	±	+	-	-	4	35	23	+	-	+	+	+	+	+	+	+	-
P-21	rod	-	+	"	+	±	+	-	-	4	35	23	+	-	+	+	+	+	+	+	+	-
P-24	rod	-	+	"	+	-	+	-	-	4	35	20	+	-	+	+	+	+	+	+	+	-
P-35	rod	-	+	"	+	-	+	-	-	4	35	20	+	-	+	+	+	+	+	+	+	-
E-19	rod	-	+	"	+	-	+	-	-	4	35	23	+	-	+	+	+	+	+	+	+	-
E-20	rod	-	+	"	+	+	+	-	-	4	35	23	+	-	+	+	+	+	+	+	+	-
E-40	rod	-	+	"	+	+	+	-	-	4	35	23	+	-	+	+	+	+	+	+	+	-
E-57	rod	-	+	"	+	+	+	-	-	4	35	20	+	±	+	+	+	+	+	+	+	-
M12F-14	rod	-	+	"	+	+	+	-	-	4	35	20	+	±	+	+	+	+	+	+	+	-
M11C-7	rod	-	+	"	+	±	+	-	-	4	35	23	+	+	+	+	+	+	+	+	+	-
MT11P-8	rod	-	+	"	+	±	+	-	-	4	35	23	+	±	+	+	+	+	+	+	+	-
MT14F-5	rod	-	+	"	+	-	+	-	-	4	35	23	+	-	+	+	+	+	+	+	+	-
MT11P-10	rod	-	+	"	+	-	+	-	-	4	35	23	+	-	+	+	+	+	+	+	+	-
MT14P-10	rod	-	+	"	+	+	+	-	-	4	35	20	+	±	+	+	+	+	+	+	+	-
MT21C-3	rod	-	+	"	+	+	+	-	-	4	35	20	+	+	+	+	+	+	+	+	+	-
MT21P-1	rod	-	+	"	+	-	+	-	-	4	35	23	+	+	+	+	+	+	+	+	+	-
MT21F-6	rod	-	+	"	+	±	+	-	-	4	35	20	+	-	+	+	+	+	+	+	+	-
MT23P-3	rod	-	+	"	+	-	+	-	-	4	35	20	+	-	+	+	+	+	+	+	+	-
MT23C-5	rod	-	+	"	+	-	+	-	-	4	35	20	+	±	+	+	+	+	+	+	+	-

Appendix 2-8. (Cont'd)

	Alcohols		Polysaccharides		Miscellaneous biochemistry										Action on pectate gel		
	Ethanol	Mannitol	Cellulose	Pectin	Arginine Dihydrolase	Catalase	Citrate	Gelatin Liquefaction	Indole	M.R.	$NO_3 \rightarrow NO_2$	Oxidase	Pigments	Starch hydrolysis	Urease	V.P.	pH 5	pH 6.8	pH 8
P marginalis																			
P-12+	+	+	-	+	+	+	+	+	-	+	+	flouresecent	-	+	-	-	+
P-21	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
P-24	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
P-35	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
E-19	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
E-20	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
E-40	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
E-57	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
M12F-14	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
M11C-7	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
MT11P-8	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
MT14F-5	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
MT11P-10	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
MT14P-10	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
MT21C-3	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
MT21P-1	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
MT21F-6	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
MT23P-3	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+
MT23C-5	+	+	-	+	+	+	+	+	-	+	+	"	-	+	-	-	+

Appendix 2-9. Physiology and biochemistry of *Serratia* isolated from alfalfa root lesions.

	Morphology				Physiology								Sugars									
					NaCl tolerance		O_2 requirement			Temperature °C												
	Shape	Gram	Motility	Flagella	5%	7%	Aerobic	Anaerobic	Spores	Min.	Max.	Opt.	Arabinose	Cellobiose	Fructose	Glucose	Lactose	Mannose	Maltose	Raffinose	Sucrose	Xylose
Serratia sp.																						
P-28	rod	-	+	Peri.	-	-	+	+	-	5	38	23	-	+	±	+	-	+	-	-	+	-
MT11P-9	rod	-	+	"	-	-	+	+	-	5	38	23	-	+	+	+	-	+	-	-	+	-
MT11F-9	rod	-	+	"	-	-	+	+	-	5	38	23	-	+	-	+	-	+	-	-	+	-
MT12P-1	rod	-	+	"	-	-	+	+	-	5	38	23	-	+	-	+	-	+	-	-	+	-

	Alcohols		Polysaccharides		Miscellaneous biochemistry								Action on pectate gel					
	Ethanol	Mannitol	Cellulose	Pectin	Arginine Dihydrolase	Catalase	Citrate	Gelatin Liquefaction	Indole	M.R.	NO_3 + NO_2	Oxidase	Pigments	Starch hydrolysis	Urease	V.P.	pH 5	pH 6.8	pH 8
Serratia sp.																			
P-28	-	+	-	-	-	+	+	+	+	+	-		Red	-	-	+	-	-	-
MT11P-9	-	+	-	-	-	+	+	+	+	+	-		"	-	-	+	-	-	-
MT11F-9	-	+	-	-	-	+	+	+	+	+	-		"	-	-	+	-	-	-
MT12P-1	-	+	-	-	-	+	+	+	+	+	-		"	-	-	+	-	-	-